MEALYBUGS OF VEGETABLE ECOSYSTEMS AND TRITROPHIC INTERACTIONS OF BRINJAL MEALYBUGS

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by

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(2017-21-014)

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KERALA, INDIA

2022

DECLARATION

I hereby declare that the thesis entitled "**MEALYBUGS OF VEGETABLE ECOSYSTEMS AND TRITROPHIC INTERACTIONS OF BRINJAL MEALYBUGS**" is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled "**MEALYBUGS OF VEGETABLE ECOSYSTEMS AND TRITROPHIC INTERACTIONS OF BRINJAL MEALYBUGS**" is a bonafide record of research work done independently by Ms. Mithra Mohan (2017-21-014) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
^{0}C	Degree Celsius
%	Per cent
CD	Critical difference
cm	Centimetre
CRD	Completely Randomised Design
DAT	Days after transplanting
et al.	And others
Fig.	Figure
g	Gram
g L^{-1}	Gram Per litre
h	Hour
ha	Hectare
ha^{-1}	Per hectare
KAU	Kerala Agricultural University
kg	Kilogram
kg ha^{-1}	Kilogram per hectare
L	litre
L^{-1}	Per litre
mg	Milligram
mL^{-1}	Per millilitre

mm	Millimetre
NBAIR	National Bureau of Agricultural Insect Resources
NS	Non -significant
No.	Number
$Plant^{-1}$	Per Plant
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
viz.	Namely

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Introduction

1. INTRODUCTION

Mealybugs are highly specialized hemipteran phytophagous insects belonging to the second largest family of Coccoidea having more than 2000 species with wide distribution around the world. Pseudococcids invaded all zoogeographic regions of the world and were mostly established in the Palearctic region and least numerous in the Neotropic region. About 158 species of mealybugs were recognized as pests with considerable economic damage to crops and ornamentals (Miller *et al.*, 2002; Ben-Dov, 2010).

Mealybugs are of great importance in economic entomology, due to their tremendous damaging potential, wide distribution pattern, broad host range, invasiveness and rapid establishment ability together with the vectoring of plant pathogens. In the past decades, the world witnessed several accidental introduction of many devastating pest species to non-native areas which resulted in bewildering impacts on the ecosystem. These highly polyphagous pests have invaded most of the geographical regions and severe pest outbreaks were recorded in different parts of the world threatening the crop production around the globe (Williams, 2004).

Management of mealybug is a tedious task due to the presence of waxy coating over the body, concealed growth habitat and clumped spatial dispersal pattern. The repetitive application of broad-spectrum chemicals led to the elimination of natural control agents, development of resistance and residue problems (Mani and Shivaraju, 2016).

A thorough understanding of the species interactions in different trophic levels of the ecosystem may aid in developing a suitable pest management strategy. The tritrophic relationships are considered as keystone interactions in the ecosystem dealing with three trophic levels *viz.*, host plant, pest and natural enemies (Agarwal and Colfer 2000). The most important prerequisite for tritrophic studies is the exact identification of target organism as each organism differs in its interactions with the environment. The taxonomic identification of

mealybugs depend on the morphological characteristics of the adult females and is often considered as a monumental task due to the phenotypic plasticity and the presence of cryptic taxa in mealybugs. Undeniably, the role of morphological taxonomy in the identification of mealybugs is colossal, but still there is a necessity for a rapid, easy and accurate method of identification of Pseudococcids that led to the advent of molecular techniques (Hebert *et al.,* 2003; Malausa *et al.,* 2011). The molecular characterization of mealybugs complement the morphological taxonomy in species confirmation in a big way.

Mealybugs are confronted by a diverse array of natural enemies that play a vital role in the regulation of the pest population. About 118 species of predators and 79 species of parasitoids of mealybugs were recorded that highlighted the importance of exploitation of biological control methods (Shylesha and Mani, 2016). A basic idea on endemic natural enemy complex in the ecosystem is of paramount importance as it provides an idea about the different species interactions in the ecosystem.

The association of ants with mealybug is a topic of interest for decades and the important subfamilies of ants in association with mealybug are Formicinae, Myrmicinae and Dolichoderinae (Mitler and Douglas, 2003). The ant-mealybug association is often considered mutualistic as ants provide protection from natural enemies, removal of excess honeydew from habitat and aid in dispersal of mealybugs and in return mealybug offer honeydew to ants (Gutierrez *et al.,* 2008). These associations should be scrutinized as they interfere with the tritrophic interactions existing in the ecosystem.

Tritrophic interactions in the ecosystem are mediated through biophysical and biochemical peculiarities of plants and a confounding array of info-chemicals. The plants exhibited several defense responses to the herbivores either through direct impact on survival and reproduction of herbivores or indirectly *via* promoting the natural enemy fauna into the ecosystem (Howe and Jander 2008). The biophysical and biochemical traits of host plants exhibited a crucial role in

maintaining these interactions *via* meddling on the activities of herbivores and natural enemies.

The info-chemical mediated tritrophic interactions in the ecosystem mainly operates through allelochemicals which include allomones, kairomones and synomones (Arimura *et al.,* 2009). Insect herbivory elicit a cascade of events in the plant system that ultimately result in the emission of volatile compounds into the atmosphere. The difference in the volatile blend of these synomones recruit specific natural enemies to the host plant. Likewise, the kairomonal compounds emanated from the prey species also act as a volatile cue for guiding natural enemies to the target pest (Turlings and Erb, 2018).

In this context, a study of tritrophic interactions is found necessary to understand the natural species interaction which may help to manipulate these interactions for better pest management. The identification of various plant or herbivore derived compounds used as cues or attractants for moderating the behaviour of natural enemies and applying inducing agents that alter the attractiveness of plants may aid in enhancement of success of biological control programmes. Breeding of crops with enhanced volatile emission and plant traits that alter the foraging behavior of herbivores and its natural enemies will also be a breakthrough in integrated pest management options.

Based on these facts, the study was conducted to meet the following objectives:

- To identify mealybugs and their natural enemy fauna in solanaceous and cucurbitaceous vegetables
- To carry out the molecular characterization of mealy bugs in solanaceous and cucurbitaceous vegetables
- To find out the tritrophic interaction of mealybug infesting brinjal.

Review of Literature

2. REVIEW OF LITERATURE

Mealybugs, 'the hard to kill insects', belonging to the family Pseudococcidae of order Hemiptera comprises of 2291 species under 274 genera. About 354 species of mealybugs under 62 genera were recorded from Southern Asia, of which 105 species were reported from India (Williams, 2004; Ben-Dov, 2010).

The name 'mealybug' was derived from the characteristic feature of waxy coating over the body which protects from unfavorable conditions (Kosztarab and Kozar, 1988). Mealybugs were often exhibited close resemblance with scale insects and were identified based on the morphological peculiarities of the adult female. Mealybugs, the phloem feeders, were found to cause alarming damage to the greenhouse crops and nurseries that lead to severe economic losses. The presence of protective wax filaments, concealed growth habitat and the high reproductive potential enable them as a noxious pest (Franco *et al.*, 2009).

Mealybugs are of great importance in the economic entomology, due to the tremendous damaging potential, wide distribution pattern, broad host range, invasiveness and rapid establishment ability together with the vectoring of plant pathogens. In the past decades, the world witnessed the accidental introduction of many devastating pest species to non-native areas which resulted in bewildering impacts on the ecosystem. These highly polyphagous pests invaded most of the geographical regions and severe pest outbreaks were recorded in different parts of the world which threaten the agriculture crop production around the globe (Williams, 2004).

2.1 CLASSIFICATION AND PHYLOGENY OF MEALYBUGS

The infraorder Coccomorpha is broadly classified into two groups, archeococcids and neococcids wherein the family Pseudococcidae belong to the group neococcids. The neococcids are characterized by the absence of abdominal spiracles, presence of apical setae in labium and adult males with simple eyes and ocelli (Koteja, 2008). Hodgson and Hardy (2013) testified the phylogenetic

relationship of infraorder Coccomorpha by adopting Bayesian method by comparing 162 characters of 269 taxa along with 29 extinct taxa and revealed the monophyletic origin of the neococcids. The origin of the infraorder Coccomorpha was estimated by total evidence approach and molecular sequencing of 73 taxa along with 43 fossil taxa that revealed the probable origin of Coccomorpha dates back to the Triassic period, of which the neococcids originated in 210 to 165 Ma and became abundant during the late Cretaceous period (Vea and Grimaldi, 2016).

Signoret (1869) classified the superfamily Coccoidea into four groups *viz.*, Diaspides, Coccides, Brachyscelides and Lecanides wherein group Coccides contains mealybugs and soft scales. Ferris (1953) separated the family Pseudococcidae from Coccidae and provided a detailed taxonomic description of 21 genera of mealybugs. Koteja (1974) divided the family Pseudococcidae into four subfamilies *viz.*, Trabutininae, Rhizoecinae, Sphaerococcinae and Pseudococcinae based on the studies on the labial structure of 84 mealybug species. The family group name Pseudococcidae was included under the Official List of Family- Group Names in Zoology with *Pseudococcus* as the type genus (Melville, 1983).

Later, the molecular phylogenetic studies revealed that only three subfamilies were present which includes, Pseudococcinae, Phenacoccinae and Rhizoecinae (Downie and Gullen, 2004). Hardy *et al.* (2008) proposed the existence of two subfamilies Phenacoccinae and Pseudococcinae and the latter divided into tribes Trabutini, Pseudococcini and Planococcini based on detailed molecular and morphological analysis of 97 species in 35 genera. Furthermore, they included the Rhizoecinae under the subfamily Phenacoccinae. The tribe Rhizoecini, previously included under the subfamily Phenacoccinae was later shifted into a new family Rhizoecidae with two subfamilies *viz.*, Rhizoecinae and Xenococcinae by Hodgson (2012).

Kaydan *et al.* (2015) conducted a phylogenetic analysis of the mealybugs of the Palearctic region disclosed that the mealybugs belong to two families *viz.*, Pseudococcidae and Rhizoecidae. They also suggested the existence of two

subfamilies in Pseudococcidae such as Pseudococcinae and Phenacoccinae with the tribes Trabutinini, Planococcini and Pseudococcini.

2.2 TAXONOMIC LITERATURE ON MEALYBUGS

The taxonomy of mealybugs was mostly depended on morphological characters of the adult females. However, efforts on taxonomic identification and understanding of evolutionary relationships based on male mealybug specimens were also practiced by several scientists across the world. Beardsley (1962) studied 30 mealybug species in Hawaii and later Afifi (1968) described 17 species of mealybugs based on characters of male specimens.

Mc Kenzie (1967) conducted meticulous research on mealybugs of California and provided detailed taxonomic notes on 193 species of mealybug along with their distribution pattern, biology and host range. A detailed study on phylogeny of Coccoidea with special emphasize on mealybugs of USSR was conducted by Danzig (1980). Later, Williams and Granara de Wilink (1992) documented 282 species of mealybugs in 49 genera in Central and South America, of which 5 genera and 62 species were new to science.

A comprehensive record on mealybugs of South Africa with a taxonomic key to 109 species of mealybugs belongs to 50 genera along with host range and distributional pattern were given by Millar (2002). Williams (2004) provided a detailed taxonomic review and illustrated about 353 species of mealybugs under 61 genera from southern Asia, of which 147 species and 6 genera were recorded for the first time in the world.

Hodgson (2020) published a monograph on neococcid scale insects based on the morphology of adult male specimens and redescriptions and illustrations were provided for 48 species. The hypogaeic (Rhizoecidae) and myrmecophilous (Xenococcidae) mealybugs were considered as separate families and also provided with a key to distinguish Pseudococcidae from Rhizoecidae.

2.3 DAMAGE IN DIFFERENT CROPS BY MEALYBUGS

Mealybugs are soft-bodied insects with elongate to oval-shaped body, covered with mealy or waxy materials and have been reported to cause potential havoc in agricultural and horticultural crops. A total of 158 species of mealybugs were recorded as important pests of crops around the world (Miller *et al.,* 2002). The damage caused by the mealybugs is associated with sap-sucking, honeydew production, injection of toxic saliva and transmission of viral diseases (Gullan and Martin, 2003). Heavy infestations often led to defoliation, distortion of the stem, stunting and ultimately the death of the plants (Hodges and Hodges, 2004).

The globalization accelerated the international trade of commodities which augmented the dispersal of mealybugs to newer regions. The polyphagous mealybug is a menace owing to its greater propensity to attack new host plants in the invaded regions that ultimately resulted in economic damage to crops (Franco *et al.,* 2009).

The invasion of the mealybug *Maconellicoccus hirsutus* (Green) resulted in severe economic impacts as they assumed to cause a loss of $ 750 million per year in the United States (Hall *et al.*, 2008). The papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink was reported to cause a potential yield loss of 91 per cent in Kenya (Macharia *et al.,* 2017). Gebregergis (2018) reported that the cotton mealybug *Phenacoccus solenopsis* Tinsley caused a severe crop loss of 100 per cent in sesame fields of Ethiopia.

The citrus mealybug *Planococcus citri* (Risso) caused yield loss up to 60 per cent in vineyards of India (Mani and Kulkarni, 2007). Dharajyoti *et al.* (2008) recorded yield loss of 40 to 50 per cent in cotton-growing tracts of Gujarat as a result of severe infestation of *P. solenopsis*. Nagrare *et al*. (2009) reported yield loss of 50 per cent due to *P. solenopsis* in all the nine major cotton-growing areas of India with an economic loss of US $16-20 million. *P. marginatus* in papaya resulted yield loss of 60 to 80 per cent in India (Mani *et al.,* 2012). A study conducted by the Centre for Plant Protection Services (CPSS, 2012) reported

economic damage to the tune of 40 per cent in cassava fields caused by *P. marginatus* in Tamil Nadu. Vijayakumari (2014) reported that *M. hirsutus* caused yield loss of 50 to 100 per cent in grapes and 30 to 50 per cent in mulberry leaf yield in India.

Several species of mealybugs are known to transmit viruses belonging to the family Caulimoviridae and Closteroviridae in various crops *viz.,* banana, grapevine, cocoa, pineapple etc. As a result of the mealybug transmitted banana streak virus, a yield loss of 49.48 per cent was recorded in India (Thangavelu *et al.,* 2000). Similarly, pineapple mealybug wilt and grapevine leaf roll disease resulted yield loss of 35 per cent and 30 to 40 per cent respectively (Sether and Hu, 2002; Maree *et al.,* 2013).

2.4 MEALYBUGS INFESTING VEGETABLES

As a result of climate changes, many mealybug species hitherto known as minor pest, attained the status of major pest in vegetables. A total of 41 species of mealybugs were documented from vegetable crops all over the world. (Tanwar *et al.,* 2007; Mani and Shivaraju, 2016). Four mealybug species were documented from the polyhouses of Kerala that were infesting vegetable crops such as tomato, brinjal, salad cucumber, cowpea and amaranths (Sreeja *et al.,* 2018).

The literature related to important mealybugs infesting vegetable crops are presented below.

2.4.1 Brinjal Mealybug *Coccidohystrix insolita* Green

Brinjal mealybug *C. insolita* was first described by Ferris (1954), later Williams (2004) provided an illustration and key to the genera *Coccidohystrix* with descriptions of two species from southern Asia.

C. *insolita* reproduce either sexually or parthenogenetically and of which latter was common. The mean nymphal period of female mealybug was 15.50 days whereas that of male mealybug was 17.02 days. The adult longevity of male mealybug was recorded as 1.44 days with a mean life cycle of 25.32 days while

adult female mealybugs with the longevity of 15.12 days with a life cycle lasts for 37.44 days (Patel, 1989).

C. insolita is prevalent in the Indian subcontinent and even distributed in other parts of the oriental region, Afrotropical and Palearctic region (Ben- Dov, 2013). Furthermore, it was recently reported in Guam and Taiwan as a potential pest of brinjal (Moore *et al.,* 2014; Chen *et al.,* 2014).

In India, *C. insolita* attained the status of a serious pest of brinjal in Kerala, Tamil Nadu, West Bengal, Bihar and several other states (Williams, 2004). Gopalakrishnapillai *et al.* (2011) recorded the infestation of *C. insolita* on tomato in Karnataka. Ben- Dov (2013) documented about 48 genera of host plants belonging to 21 families infested by *C. insolita,* of which Solanaceae and Malvaceae were recorded as the predominant families. A total of nine host plants were recorded from Kerala *viz.,* brinjal, red gram, *Commelina* sp., *Cyclea* sp., *Clitoria* sp, *Physalis* sp., *Sida* sp., *Solanum* sp. (Jose, 2017). Nagalakshmi (2019) documented brinjal, tomato and *Abutilon* sp. as host plants of *C. insolita* from Karnataka.

2.4.2 Cotton Mealybug *Phenacoccus solenopsis* Tinsley

P. solenopsis was first described by Tinsley (1898) on the adult female collected from stems of *Boerhavia spicata* Choisy and *Kallstroemia californica* (S. Watson) Vail and *Atriplex canescens* (Pursh) Nutt. in New Mexico, USA and it was named as *P. solenopsis* by Ferris (1950). It was redescribed by Williams and Granara de Wilink (1992) and Kosztarab (1996) from different regions of the world. Hodgson *et al*. (2008) described all the immature stages of *P. solenopsis* with illustrations based on the specimens collected from India and Pakistan.

The mealybug *P. solenopsis* is ovo-viviparous with mean life duration of 43.4 days with a mean adult female longevity of 24.8 days in okra. The average fecundity rate is 385.5 eggs with a survival rate of 91.9 per cent. The total life cycle of male mealybug lasts for 17.4 days. (Sahito *et al.,* 2010).

P. solenopsis, a highly invasive polyphagous pest, was first recorded from Asia in Pakistan (Abbas *et al.*, 2005) and India (Jhala *et al.*, 2008) which were recognized as cotton hubs of southern Asia. *P. solenopsis* was found to be distributed in about 50 countries in Asia, Africa, Oceania, South America, North America and Europe (Garcia Morales *et al.*, 2016).

A severe incidence of *P. solenopsis* was noticed in Pakistan from 154 plants belongs to 53 families which include Malvaceae, Solanaceae, Asteraceae, Euphorbiaceae, Amaranthaceae and Cucurbitaceae. Severe economic damage was noted in seven crops *viz.*, cotton, tomato, brinjal, okra, sesame, sunflower and China rose which caused the death of the plant (Arif *et al.*, 2009). It was also recorded on nearly 204 host genera under 64 families around the world (Garcia-Morales *et al.*, 2016). Capinera (2020) documented 200 host plants of *P. solenopsis*, of which the majority belongs to the families Asteraceae, Solanaceae, Malvaceae and Fabaceae with important vegetable hosts *viz.*, okra, tomato, brinjal, pumpkin, chilli, potato and gourds.

Vennila *et al.* (2010) recorded a total of 84 host plants across 28 families in India, of which Asteraceae, Leguminaceae, Malvaceae and Solanaceae represented about 50 per cent of the host plants. Nagrare *et al.* (2012) reported 166 host plants under 51 families contains vegetables, field crops, fruit crops, spices and weeds. *P. solenopsis* was documented as the predominant mealybug in the vegetable ecosystem and found to be damaging tomato, brinjal, chilli, okra and pointed gourd throughout the year in India (Halder *et al.*, 2015).

P. solenopsis was documented from 40 host plants in Kerala, in which Asteraceae, Malvaceae, Solanaceae and Amaranthaceae contributed to the maximum share (Padmanabhan, 2017). *P. solenopsis* exhibited a wide host range of 202 plants belongs to 55 families that comprised of vegetables, fruits, ornamentals, field crops and weeds across the world (CABI, 2020).

2.4.3 Papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink

P. marginatus was presumed to be originated in Central America, where it never attained the status of a major pest owing to its rich natural enemy fauna. A detailed description with suitable illustration, based on adult female collected from the neotropical region (Belize, Costa Rica, Guatemala and Mexico) was provided by Williams and Granara de Willink (1992). A comprehensive description of immature stages of mealybug along with adult male was given by Miller and Miller (2002).

The male mealybug with a total life cycle of 26.6 days and that of female is 39.7 days. . The adult female longevity was recorded as 17.6 days whereas male longevity was 2.7 days in papaya (Chellappan *et al.,* 2013a).

P. marginatus was first reported in India from Coimbatore district of Tamil Nadu in 2008 on papaya (Muniappan *et al.,* 2008) and later invaded to Kerala in 2009 as reported by Krishnakumar and Rajan (2009). This highly invasive mealybug had broadened its distribution over 52 countries across the globe during the past decades. Recently it was reported in Western Palearctic region (Mendel *et al.,* 2016) and Kenya (Macharia *et al.,* 2017).

A total of 133 plant species under 48 families were recorded as host plants of papaya mealybug which include pulses, oilseeds, fiber crops, vegetables and fruit trees, of which the most preferred families were Malvaceae, Solanaceae, Asteraceae and Euphorbiaceae (Sakthivel *et al.,* 2012). It is a highly polyphagous exotic pest with wide host range, documented from 95 plants under 39 families, majority of which belong to Euphorbiaceae, Fabaceae, Asteraceae, Apocynaceae, Malvaceae and Solanaceae in Kerala (Chellappan *et al.,* 2013b). Garcia-Morales *et al.* (2016) reported a total of 137 plant genera belong to 49 families as host plants of *P. marginatus* in the world.

2.4.4 Pink hibiscus mealybug *Maconellicoccus hirsutus* (Green)

M. hirsutus was first described in India by Green (1908) as *Phenacoccus hirsutus* based on a specimen collected from an undetermined shrub. Later, Ezzat (1958) described the genus *Maconellicoccus* under the tribe Planococcini and designated *P. hirsutus* as the type species of the genus. It is believed to be native of southern Asia and invaded about 91 countries in all biogeographic regions across the globe (Williams, 1996; Chong, 2009). It was considered as one of the most prevalent mealybug infesting economically important crops in India (Mani *et al.,* 2011).

The mealybug *M. hirsutus* completed the life cycle within a period of 42.0 days. The adult female longevity was 16.6 days whereas male longevity was 1.2 days in grapevine (Amala, 2015).

M. hirsutus is highly polyphagous with a host record of 293 species belongs to 75 families which include economically valuable crops such as fruits, vegetables, field crops and ornamental plants (Meyerdirk *et al.,* 2001). About 223 genera of plants in 78 families were recorded as the host plants of *M. hirsutus* in the world, in which Malvaceae was considered as the most preferred host (Garcia-Morales *et al.*, 2016). The most susceptible vegetable hosts of *M. hirsutus* were bean, carrot, pumpkin, okra, cowpea and tomato (Capinera, 2020).

2.4.5 Striped mealybug *Ferrisia virgata* (Cockerell)

F. virgata was first recognized by Cockerell (1893) as *Dactylopius virgatus* based on the specimens collected from Jamaica and later Ferris (1919) described and illustrated the main identifying characters of the species. Fullaway (1923) established a genus-group *Ferrisia* which was erroneously considered as a homonym of *Ferrisiana* by Takahashi (1929) and later Morrison and Morrison (1966) removed the replacement name. It is believed to be a native of the New World and extended its range to 102 countries around the world (Garcia-Morales *et al.,* 2016).

The mealybug, *F. virgata* completed the life cycle within 48.2 days. The adult longevity recorded as 30.08 days with a fecundity rate of 109 to 180 in *Coccinia* sp. (Vigneswaran *et al.,* 2016).

F. virgata, a cosmopolitan pest, documented from various economically important crops such as tomato, brinjal, cotton, citrus, papaya, sweet potato, cassava and coffee (Schreiner, 2000). Mani *et al*. (2011) recorded a wide host range in India, which includes citrus, guava, custard apple, tuberose, acalypha, jackfruit, ixora, mango and pomegranate. *F. virgata* was recorded on 207 plant genera belongs to 78 families and most of which were included under Fabaceae and Euphorbiaceae (Garcia-Morales *et al.,* 2016).

2.4.6 Other mealybugs infesting vegetables

About nineteen species of mealybugs were recorded from tomato whereas brinjal and okra were infested by 14 and 6 species respectively (Mani and Shivaraju, 2016).

The oriental mealybug *Planococcus lilacinus* Cockerell was first described by Morrison (1920) followed by Cox (1989) and Williams and Granara de Willink (1992) based on the adult female while Tang (1992) described the species based on the adult male specimen. It was recognized as a serious pest on Chow-chow in Bangalore (Krishnamoorthy and Mani, 1998). It was also recorded as a severe pest on cauliflower, which resulted in stunted growth and reduced curd formation (Loganathan and Suresh, 2001). *P. lilacinus* is distributed over 34 countries with a wide host range of 71 plant genera belongs to 36 families (Garcia-Morales *et al.*, 2016).

The Passion vine mealybug *Planococcus minor* (Maskell), a mealybug of old world origin, first described by Williams and Watson (1988) and later by Tu *et al*. (1988) and Williams (2004). It is considered as an economically important pest in several vegetable crops *viz.,* celery, ash gourd, pumpkin and soybean in Taiwan (Ho *et al.,* 2007). This polyphagous pest invaded about 64 countries across the world with a host range of 196 genera belongs to 73 families

which include brinjal, tomato, amaranths, cabbage, radish and cucurbits (Garcia-Morales *et al.*, 2016).

The citrus mealybug *P. citri*, a native of the old world, was described initially as *Dorthesia citri* (Risso, 1813) from the specimens collected from citrus in France. Later, it was transferred to the genus *Planococcus* by Ferris (1950) and detailed morphological illustrations were provided by Afifi (1968) based on male specimens and Cox (1989) and Willliams (2004) based on the adult female. It invaded almost 161 countries and recorded with a wide host range of 199 genera in 84 families (Garcia-Morales *et al.*, 2016). A total of 70 host plants were recorded across the world, of which majority belongs to Fabaceae, Rutaceae and Solanaceae (CABI, 2020).

The Jack Beardsley mealybug *Pseudococcus jackbeardsleyi* Gimpel and Miller presumed to be originated in the new world, first described by Gimpel and Miller (1996). It was recorded from 22 plant species in Asia including tomato, ivy gourd and potato (Williams, 2004). A total of 112 plant genera in 52 families which includes Cucurbitaceae, Solanaceae and Malvaceae were recorded as hosts of *P. jackbeardsleyi* (Garcia-Morales *et al.*, 2016).

The long-tailed mealybug *Pseudococcus longispinus* (Targioni Tozzetti) was first described and illustrated by Zimmerman (1948) followed by subsequent revisions by Ferris (1950), Beardsley (1960), Cox (1987), Lit and Caliliung (1994) and Willliams (2004). It is distributed over 118 countries which include tropical, subtropical and temperate regions across the world with a wide host range of 167 genera belongs to 83 families with reports from pumpkin, chilli and brinjal (Garcia-Morales *et al.*, 2016). Sreeja *et al.* (2018) recorded *P. longispinus* as an important pest of summer squash in polyhouses of Kerala.

2.5 NATURAL ENEMIES OF MEALYBUGS

Natural enemies play a vital role in the management of the mealybug population in an ecosystem.

2.5.1 Predators

The major predators of mealybugs belong to the families Coccinellidae, Hemerobiidae, Chrysopidae, Lycaenidae, Syrphidae, Drosophilidae and Cecidomyiidae. A total of 118 species of predators were recorded from mealybugs, which signified the importance of biocontrol agents in the population regulation of mealybugs around the world (Shylesha and Mani, 2016).

A survey conducted at Turkey revealed a rich natural enemy complex of 23 species belonging to the families *viz.,* Coccinellidae, Chrysopidae, Hemerobiidae and Chamaemyiidae that predated on 22 different mealybugs infesting crops (Kaydan *et al.,* 2006). A field study conducted at Thailand disclosed a total of ten species of predators namely *Cryptogonus orbiculus* (Gyllenhal), *Sasajiscymnus quinquepunctatus* (Weise), *Scymnus quadrillum* Motschulsky, *Scymnus* sp. and *Stethorus* sp. belongs to the family Coccinellidae and two neuropterans such as *Chrysoperla* sp. and *Mallada basalis* (Walker) along with the lycaenid predator *Spalgis epius* (Westwood) from the papaya mealybug *P. marginatus* (Saengyot and Burikam, 2011).

Twelve predatory insect fauna were recorded from Pseudococcidae of Iran, which include different genera namely *Coccinula*, *Diomus, Exochomus, Hyperaspis, Nephus* and *Oenopia* under the family Coccinellidae and genus *Dicrodiplosis* in Cecidomyiidae along with the neuropterans *Chrysoperla sillemi* (Esben-Petersen) and *Wesmaelius* sp. (Jalilvand *et al.,* 2014). Gagne and Jaschhof (2014) reported that predatory gall midges of the genera *viz., Diadiplosis, Dicrodiplosis, Triommata* and *Megommata* were recognized as competent predators of mealybugs owing to its proficiency in locating the prey and were less prone to the intra-guild predation. Attia and Awadallah (2016) identified six species of predators belongs to the family Coccinellidae, Cecidomiidae, Chrysopidae, Hemerobiidae and Anthocoridae from the cotton mealybug *P. solenopsis* in Egypt.

The pink mealybug *M. hirsutus* was predated by an array of 30 insect species under 11 families in five orders, whereof the coccinellid *Cryptolaemus montrouzieri* Mulsant was the predominant predator (Mani, 1989). Ram and Saini (2010) reported that five species of coccinellids were predated on the mealybug *P. solenopsis* in cotton-growing tracts of Haryana, which includes *Brumoides suturalis* (Fabricius), *Scymnus coccivora* Ayyar, *Cheilomenes sexmaculata* (Fabricius), *Nephus regularis* Sicard and *Hippodamia variegata* (Goeze), of which *B. suturalis* was recorded as the most abundant species.

About 11 species of predators were recorded on papaya mealybug *P. marginatus*, in which *S. epius* was considered as a major predator in southern India (Thangamalar *et al.*, 2010; Mani *et al.*, 2012). Twelve species of predators belong to four families were documented from *P. solenopsis*, of which Coccinellidae and Chrysopidae were recognized as the major families involved in the management of mealybugs in horticultural crops (Fand and Suroshe, 2015).

A study conducted by Amala (2015) recorded a total of ten predator species belonging to two families such as Coccinellidae and Chrysopidae from *M. hirsutus* in the grape-vine ecosystem of Maharashtra and Tamil Nadu. Exploration of natural enemies on *M. hirsutus* disclosed a total of seven predators *viz., Scymnus nubilus* Mulsant, *N. regularis*, *S. coccivora*, *C. montrouzieri* of Coccinellidae and *Leucopis* sp. (Chamaemyiidae) and *Cacoxenus (Gitonides) perspicax* Knab (Drosophilidae) along with *S. epius* in Karnataka (Prasanna and Balikai, 2015).

Coccinellid beetles, *Scymnus* (*Pullus*) sp. and *Horniolus* sp. were recorded as predators of root mealybug *Geococcus citrinus* Kuwana in banana and *F. polysperes* in pepper respectively in Kerala (Smitha, 2007; Najitha, 2016). Vidya and Bhaskar (2017) identified four genera of coccinellids namely *Scymnus, Cryptolaemus, Horniolus* and *Nephus* under the tribe Scymini as predators of mealybugs in Kerala. Among these, *S. (Pullus) coccivora* was recorded as the most dominant one with the widest host range of six mealybugs. A survey

conducted in Kerala to identify the natural enemies associated with *D. brevipes* in pineapple disclosed that *Scymnus* sp. was the dominant predator with a relative abundance of 68.75 per cent followed by *S. epius* (17.61%) and *C. perspicax* (7.39 %) (Manjushree *et al.*, 2019).

2.5.2 Parasitoids

Mealybugs are parasitized by an array of insects belongs to the hymenopteran families namely Encyrtidae, Aphelinidae, Pteromalidae, Platygastridae, Braconidae, Eulopidae, Signiphoridae and Eucoilidae. The predominant parasitoids of mealybugs were included under three families *viz.*, Encyrtidae, Aphelinidae and Platygastridae which play a vital role in population regulation of mealybugs in natural ecosystems. A total of 79 species of parasitoids were recorded from different mealybugs, of which the family Encyrtidae accounts for a major role in pest management (Shylesha and Mani, 2016).

A list of parasitoids infesting mealybugs is given in Table 1.

2.6 ANTS ASSOCIATION WITH MEALYBUGS

The association of ants has been pondered as a topic of research over decades and were mostly concentrated on the trophobiotic relationship of ants with honeydew-producing hemipterans especially mealybugs (Holldobler and Wilson 1990). The important subfamilies of ants in association with Hemiptera are Formicinae, Myrmicinae and Dolichoderinae (Mitler and Douglas, 2003).

The honeydew offered by the mealybug acts as a source of nutrition which contributes about more than half of the diet of many ant species. In rare cases, ants regulate the population of mealybugs by consuming it, to adjunct the protein needs of the body (Mittler and Douglas 2003). The ant-tended mealybug colonies also obtained certain benefits *viz.*, provision of shelter, removal of excess honeydew from habitat and transportation which favored the establishment and better survival of the mealybug population. Besides, ants disrupt the movement of

Table 1. Parasitoids reported from mealybugs infesting vegetables

Sl. No	Parasitoids	Family	Host insect	Location	Reference
1	*Coccophagus* sp. *Marietta picta*	Aphelinidae	*Planococcus vovae*	Turkey	Kaydan *et al.* (2006)
	Anagyrus aligarhensis *Anagyrus pseudococci* *Anagyrus schoenherri* *Coccidoxenoides perminutus* *Ericydnus robustior* *Leptomastidea matritensis* *Leptomastix flava* *Mayridia pulchra* *Stematosteres* sp. *Tetracnemoidea* sp.	Encyrtidae	*Phenacoccus pumilus* *Phenacoccus citri* *Planococcus ficus* *Phenacoccus aceris* *Phenacoccus ferulae*		
	Allotropa mecrida	Platygastridae	*Phenacoccus aceris*		
	Eunotus sp. *Eunotus acutus* *Pachyneuron concolor*	Pteromalidae	*Phenacoccus avenae*		
2	*Adelencyrtus coxalis* *Aenasius advena* *Aenasius bambawalei* *Aenasius indicus* *Alamella flava* *Anagyrus chrysos* *Anagyrus dactylopii* *Anagyrus gracilis* *Anagyrus indicus* *Anagyrus kamali* *Anagyrus mirzai* *Blepyrus insularis* *Gyranusoidea flava* *Gyranusoidea indica* *Leptomastix nigrocincta* *Mahencyrtus assamensis* *Neoplatycerus tachikawai* *Praleurocerus viridis* *Rhopus nigroclavatus*	Encyrtidae	*Brevennia rehi* *Ferrisia virgata* *Phenacoccus solenopsis* *Maconellicoccus hirsutus* *Coccidohystrix insolita* *Rastrococcus iceryoides*	Tamil Nadu	Nalini and Manickavasagam (2016)
3	*Aenasius bambawalei*	Encyrtidae	*Phenacoccus solenopsis*	Punjab Haryana Rajasthan Gujarat Maharashtra Madhya Pradesh	Tanwar *et al*. (2011)
	Promuscidea unfasciativentris	Aphelinidae			
4	*Aenasius bambawalei* *Leptomastix* sp. *Paranathrix tachikawai* *Anagyrus* sp.	Encyrtidae	*Phenacoccus solenopsis*	Delhi, Maharashtra	Suroshe *et al.* (2013)

5	*Coccidoxenoides perminutus Anagyrus dactylopii* *Leptomastix dactylopi*	Encyrtidae	*Maconellicoccus hirsutus* *Planococcus citri*	Maharashtra	Amala (2015)
6	*Coccophagus peudococci* *Coccophagus* sp. *Promuscidea unfasciativentris* *Euryischomyia* sp.	Aphelinidae	*Maconellicoccus hirsutus*	Karnataka	Prasanna and Balikai (2015)
	Aenasiusbambawalei *Leptomastix dactylopii* *Leptomastix lyciae*	Encyrtidae			
	Oomyzus sp.	Eulophidae			
	Centiste sp.	Braconidae			
	Metastenus concinnus	Pteromalidae			
7	*Acerophagus gutierreziae*	Encyrtidae	*Phenacoccus solenopsis*	Egypt	Attia and Awadallah (2016)
	Chartocerus dactylopii	Signiphoridae			
8	*Anagyrus indicus* *Anagyrus agraensis* *Anagyrus loecki* *Anagyrus qadrii* *Coccidoctonus terebratus* *Leptomastidea shafeei* *Leptomastix gunturiensis* *Leptomastix dactylopii*	Encyrtidae	*Nipaecoccus* sp. *Phenacoccus indicus* *Planococcus citri* *Planococcus lilacinus* *Coccidohystrix insolita* *Phenacoccus solenopsis* *Paracoccus marginatus* *Pseudococcus longispinus* *Phenacoccus divaricatus*	Karnataka	Joshi *et al.* (2017)
9	*Anagyrus arizonensis* *Anicetus* sp. *Prochiloneurus* sp	Encyrtidae	*Phenacoccus solenopsis*	Kerala	Padmanabhan (2017)
	Myiocnema comperei	Aphelinidae			
10	*Acerophagus papayae* *Pseudleptomastix mexicana*	Encyrtidae	*Paracoccus marginatus*	Tamil Nadu Pudhucherry	Ayyamperumal and Manickavasagam (2018)
11	*Chartocerus* sp.	Signiphoridae	*Dysmicoccus brevipes*	Kerala	Manjushree *et al.* (2019)

natural enemies, thus provide protection from natural control mechanism (Gutierrez *et al.,* 2008).

Ants belonging to the genera *Camponotus, Crematogaster* and *Pheidole* were recorded to tend the mealybug *Phenacoccus manihoti* Matile-Ferrero and created adverse impacts on the biological control programmes in Ghana (Cudjoe *et al.,* 1993). A total of 28 ant species were found to be in association with mealybugs causing wilt disease of pineapple, wherein *Pheidole* and *Solenopsis* were recorded to be the dominant genera (Jahn *et al.,* 2003). Beltra *et al.* (2017) recorded three ant species in association with the mealybug *Planococcus ficus* (Signoret) in the vineyards of Spain which comprises of *Lasius grandis* (Forel), *Pheidole pallidula* (Nylander) and *Plagiolepis schmitzii* (Forel). *M. hirsutus* exhibited a trophobiotic association with 46 genera of ants, belongs to 17 species under five subfamilies, the majority of which belongs to the Myrmicinae. The genera, *Crematogaster, Campanotus* and *Pseudomyrmex* were noted with the highest number of interactions with *M. hirsutus* on cocoa plantations in Brazil (Marques *et al.,* 2018).

Tanwar *et al.* (2007) recorded that *P. citri* was tended by the ant genera *Oecophylla, Anoplolepis* and *Crematogaster* in *Hibiscus* sp. A total of four ant species *viz. Monomorium indicum* Forel, *Solenopsis geminata* (Fabricius), *Tapinoma sessile* (Say) and *Camponotus compressus* (Fabricius) were recorded to tend the mealybug *M. hirsutus* in Tamil Nadu. The attendant ants dispersed the pest and also deterred the natural enemies away from the mulberry crop (Mahimasanthi *et al.,* 2014). Eight species of ants were recorded to be in association with the mealybug *P. marginatus*, of which the ant species *S. geminata* was considered as the most dominant attendant ant in Karnataka (Gowda *et al.,* 2014). Singh and Kumar (2017) reported that the mealybug *P. solenopsis* was attended by three ant species *viz., Monomorium pharaonis* Linnaeus, *C. compressus* and *Tapinoma*

melanocephalum Fabricius, which negatively affect the population of parasitoid *Aenasius bambawalei* Hayat in different host plants.

Venkataramaiah and Rehman (1989) reported that the ant species *viz.*, *Crematogaster* sp., *Tapinoma* sp., *Technomyrmex* sp., *Paratrechina* sp., *Acropyga* sp. and *Plagiolepis* sp. were in association with mealybugs of coffee in Kerala and Karnataka. Devasahayam *et al.* (2010) recorded that two ant species such as *Anoplolepis* sp. and *Technomyrmex* sp. were in association with the mealybugs at the root zone of pepper. A total of four ant species such as *Anopolepis gracilipes* Smith, *Crematogaster rogenoferi* Mayr, *Lephomyrmex quadrispinsus* Jerdon and *Paratrechina* sp. were found to be in association with the root mealybugs such as *Formicococcus polysperes* Williams and *Dysmicoccus brevipes* Cockerell of pepper in Kerala (Najitha, 2016). Two species of ants were recognized as attendant ants of pineapple mealybug *D. brevipes* in Kerala, which includes *Camponotus mitis* (Smith) and *T. albipes* (Manjushree *et al.*, 2019).

2.7 MOLECULAR CHARACTERIZATION OF MEALYBUGS

Taxonomic identification of members of the family, Pseudococcidae requires considerable expertise as it is based on the cuticular structures of the slide-mounted adult female viewed under a high definition microscope which is a tedious and time-consuming process (Millar, 2002). Furthermore, the phenotypic variations arise as a result of environmental conditions, presence of cryptic taxa and difficulty in identification of immature stages and males made the taxonomy of mealybug a monumental task. The exact identification of mealybug is necessary when dealt with the timing of pest management strategies, implementation of biological control programmes and also in the regulation of quarantine pests. In this scenario, the necessity for an adjunct tool which provides a highly accurate, rapid and convenient method for taxonomic separation of the family Pseudococcidae, accelerated the advent of molecular techniques such as DNA barcoding approach (Hebert *et al.,* 2003; Malausa *et al.,* 2011).

Studies concerted on the use of DNA barcoding to untangle the species complex in mealybugs revealed that mtDNA COI gene is considered as the main barcode region owing to its maternal inheritance and high stability (Hebert *et al.,* 2003). However, the universal primers used to amplify this particular region was not performing well in many species of Pseudococcidae. Malausa *et al.* (2011) identified five efficient genetic markers to amplify the two regions of the mitochondrial cytochrome oxidase I gene, 28S-D2, ITS2 locus and the rpS15-16S region of the endosymbiont *Tremblaya princeps.* They reported that these markers can be considered as a proficient tool for understanding the cryptic mealybug taxa (28S-D2), the phylogenetic relationship of mealybugs (all markers except ITS2), population genetics and DNA barcoding (ITS2 and COI regions).

Beltra *et al.* (2012) analyzed 33 mealybugs in Spain, which revealed the existence of 16 multi-locus haplotypes in ten different species and provided sequence data for three species which were not sequenced yet. The phylogenetic tree disclosed that the genera *Pseudococcus* is paraphyletic whereas *Phenacoccus* and *Planococcus* belong to the monophyletic groups. Correa *et al.* (2012) conducted a study on mealybug populations collected from Chilean vineyards disclosed the presence of 12 multilocus genotypes belongs to *Pseudococcus viburni* (Signoret), *Pseudococcus meridionalis* Prado and *Pseudococcus cribata* Gonzalez. The study also pointed out that *P. viburni* and *P. cribata* exhibited high genetic variability which confirmed the origin of these two species in South America. Zheng *et al.* (2019) identified that the COI gene fragment of 500 bp can be used for the precise identification of the members of Pseudococcidae with a success rate of 97.84 per cent by analyzing the intra and interspecific divergences and estimating the barcoding gap of 21 species of mealybugs in China.

Species-specific PCR (SS-PCR) with species-specific primers were considered as a budding tool in molecular characterization of mealybugs, which confirm the species identity merely based on the presence of a particular band in gel electrophoresis. A multiplex PCR method was devised in South Africa to

discern *Planococcus ficus*, *P. citri* and *P. longispinus*. (Saccaggi *et al.*, 2008). Tian *et al*. (2013) utilized SS-COI primers for the detection of *P. solenopsis* whereas Wang *et al*. (2019) devised SS-COI primers PMSSZW-1F and PMSSZW-1R for the easy detection of *P. manihoti* in cassava.

2.8 TRI-TROPHIC INTERACTIONS WITH RESPECT TO MEALYBUGS

Tri- trophic relations are mainly mediated through plant morphological traits and confounding array of info-chemicals. The plants confront the herbivores directly through creating adverse effects on survival and reproduction of herbivores either by biophysical or biochemical traits and indirectly *via* promoting the natural enemy fauna using semiochemicals into the ecosystem (Howe and Jander, 2008).

2.8.1 Host Plant Factors Mediating Tri-trophic Interactions

The impact of a plant trait on the natural enemy mainly depends on the physical, biochemical and behavioural relationship present amid the host plant, herbivore and its natural enemy (Kennedy, 2003). Host plant characters impart a negative impact on the fitness of natural enemies either through direct exposure to the toxic plant metabolites in the body of the host insect, meddling on the activity of entomophages or indirectly by reduced host size and quality (Ode, 2006).

2.8.1.1 *Role of Biophysical Traits of the Host Plant in Mediating Interactions among Mealybugs and Its Natural Enemies*

Plant morphological traits perform a vital role in sustaining tri-trophic relations in an ecosystem by interfering on the activity of herbivores and its natural enemies. The morphological characters of plants alter the oviposition and feeding behaviour of insect pests, thus wielded a critical role in host plant resistance (Rebe *et al.*, 2004). The information on the impact of host plant morphological characters on the natural enemy population of mealybugs was meagre. Even though, the leaf surface traits that influence the mealybug population may also affect the population of natural enemies.

Johnson-Cicalese *et al.* (1998) evaluated the turf-type buffalo grass genotypes to identify the resistance towards mealybug *Tridiscus sporoboli* (Cockerell) and *Trionymus* sp., revealed that trichome density exerted a positive impact on the establishment of mealybug population. An experiment conducted by Shahid *et al*. (2012) disclosed that trichome density and trichome length favoured the population buildup of cotton mealybug *P. solenopsis* in different host plants. Evaluation of five mulberry genotypes against the mealybug *M. hirsutus* disclosed that the cultivar V1 and S36 were found to be preferred by the mealybug and the trichome density of the tested genotypes exhibited a significant positive correlation with the population of mealybugs (Mahimashanthi, 2014).

Heidari (1999) recorded that trichome density altered the host searching capability of coccinellid predators on mealybug *P. viburni* by reducing the walking speed over the leaf surface. Kennedy (2003) reported that the presence of glandular trichomes imparted an undesirable impact on the natural enemies by interfering the movement which led to poor searching efficiency and also resulted in entrapment and death of the entomophages on the toxic sticky secretion of trichomes. Balakrishnan *et al.* (2006) reported that the trichome density of cotton genotypes exhibited a negative correlation with oviposition and predation of *Chrysoperla carnea* (Stephens).

Shahid *et al.* (2012) reported that leaf size and leaf width exerted significant negative correlation with the population of mealybug *P. solenopsis* in cotton. Shahid (2016) revealed that the different instars of cotton mealybug *P. solenopsis* exhibited a significant positive correlation with leaf area and leaf thickness in different host plants.

The life-history parameters of the coccinellid predator, *Exochomus flaviventris* Mader, was influenced by the host plants of mealybug, *P. manihoti*. Plant morphological traits *viz.,* leaf toughness and leaf wax content may be involved in mediating the interactions involving the host plant, mealybug and natural enemy (Le Ru and Mitsipa, 2000). An experiment conducted by Cloyd

and Sadof (2000) in citrus mealybug *P. citri* and its parasitoid *L. dactylopii* in coleus revealed that plant morphological traits such as leaf surface area, number of leaves, plant height, number of branches and plant size were negatively correlated with the attack rate of the parasitoid. Garcia and O' Neil (2000) recorded that a higher predation rate on *P. citri* was shown by *C. montrouzieri* on plants with lower leaf number, plant height and leaf area, of which leaf area was considered as the key factor governing the predatory rates.

The spatial heterogeneity of the host plants interfered both predator and prey by either providing a refugee for the prey or by creating hindrances on the activity of predators and prey. A study performed to identify the effect of plant structural factors on the functional response exhibited by the coccinellid predator *C. montrouzieri* on a diet of *P. citri*. The results revealed that a higher rate of predation was exhibited by *C. montrouzieri* on chilly plants with simple morphological structures such as fewer branches and leaves, which denoted that the chance of encountering a prey was more frequent in plants with less complex architecture (Zhu, 2016).

2.8.1.3 *Role of Biochemical Traits of the Host Plant in Mediating Tritrophic Interactions among Mealybugs and Its Natural Enemies*

An experiment was conducted to screen the banana cultivars against root mealybugs in Kerala revealed that the cultivars, Palayankodan and Kodappanillakunnan with the lowest population of mealybug and with the highest phenol content. (Smitha, 2007). Eid *et al*. (2011) reported that the population of mealybug *Saccharicoccus sacchari* (Cockerell) exhibited a significant positive correlation with total proteins and reducing sugars in different sugarcane cultivars while total phenol showed a negative correlation with mealybug. An experiment conducted by Yakoub (2012) on pink sugarcane mealybug, *S. sacchari* in different sugarcane cultivars revealed that the total carbohydrates, non-reducing sugars, crude protein, ash and nitrogen content showed a significant positive correlation with mealybug population.

The biochemical constituents *viz.,* higher phenolic concentration, increased activity of polyphenol oxidase and peroxidase enzymes present in brinjal plant impart resistance to mealybug *P. marginatus.* Furthermore, total carbohydrates, total sugars and total chlorophyll content were higher in susceptible cultivar CO2 while low moisture content and higher ash content were noted in resistant genotype *Solanum viarum* Dunal (Janaki and Suresh, 2012). A study conducted to screen the grapevine cultivars against *M. hirsutus*, reported that the biochemical parameters *viz.*, reducing sugars, total proteins and amino acids of nodes and leaves exhibited a significant positive correlation with mealybug incidence whereas phenol content exhibited a negative correlation (Amala, 2015). Nisha and Kennedy (2017) recorded that *P. marginatus* exhibited a positive association with total carbohydrates and total sugar present in the host plant and a negative correlation with phenol and tannin content.

The study on the role of oxidizing enzymes mediated host plant resistance in cotton revealed that polyphenol oxidase, polyphenol peroxidase and catalase activity was higher in cotton cultivars susceptible to the mealybug *P. solenopsis* (Ghule *et al.*, 2011). The investigation on molecular and biochemical changes due to mealybug infestation revealed that phenol content, terpenoid content, defensive enzymes such as phenyl ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) increased with mealybug feeding time in cotton (Shafique *et al.*, 2014). Nagrare *et al.* (2017) studied the biochemical changes in the cotton plants mediated by mealybugs disclosed that total protein content and total phenol content may increases whereas carotenoid and chlorophyll concentration decreases as a result of infestation by *P. solenopsis.*

Elanchezhyan *et al.* (2008**)** evaluated 25 brinjal cultivars to identify the preference shown by insect pests including the eggplant mealybug *C. insolita* and its natural enemies in Tamil Nadu. The study revealed that total sugars, chlorophyll and moisture content showed a positive correlation whereas total phenols and ash content exerted a negative correlation with the population of pests. Among the tested genotypes, highly susceptible cultivars, Bejo Sheetal and

Pusa hybrid-6 harboured the highest population of coccinellids, syrphids and spiders.

A study conducted on *M. hirsutus* in mulberry and its natural enemies revealed that the population of mealybug exhibited a significant positive correlation with the total amino acid content, total soluble sugars and chlorophyll content of leaves whereas phenol content and protein content showed a significant negative correlation. The predatory population exhibited a significant positive correlation with total soluble sugars and protein content of the leaf while phenol content and chlorophyll content exerted a negative correlation. The population of predators were not influenced by the amino acid content present in the leaf (Mahimasanthi *et al.,* 2014).

2.8.2 Info-chemicals mediating tri-trophic interactions

Info-chemicals are involved in intraspecific and interspecific communication, and the latter plays a vital role in maintaining tritrophic interactions. The interspecific communication is mainly operated through allelochemicals, compounds that were produced by an organism which alter the behavior of organisms of another species (Dicke and Sabelis, 1988). Allelochemicals are of different types, of which synomones and kairomones are mainly involved in the tritrophic relations in an ecosystem.

2.8.2.1 *Role of Synomones in Mediating Tritrophic Interactions among Mealybugs and Its Natural Enemies*

Plants that were infested by herbivores released plant volatiles that communicates with the natural enemies about the presence of herbivore on the plants and benefits the plant by attracting the natural enemies of pests and aids natural enemies by orienting it to the suitable host (Turlings *et al.,* 1990).

An experiment conducted to identify the response of *Apoanagyrus (Epidinocarsis) lopezi* De Santis, a parasitoid of cassava mealybug *P. manihoti,* towards the plant volatiles emitted from the cassava leaves revealed that mealybug infested plants attracted more parasitoids than un-infested plants.

The herbivore-induced synomones produced in mealybug infested plants acted as long-distance cues to guide the parasitoids. Furthermore, the release of volatile chemicals was not restricted to the infested region, rather it emanated from the whole plant surface (Souissi *et al.,* 1998).

Bertschy *et al.* (2001) conducted a study to elucidate the role of mealybug induced volatiles in attracting the parasitoids such as *Aenasius vexans* Kerrich and *Apoanagyrus diversicornis* Howard to the cassava plants. The olfactometer studies recorded a higher attraction of parasitoids to the odours emanated from mealybug infested plants over the healthy plant and odours emitted from the mealybug itself. This experiment also pointed out that the herbivore-induced synomonal compounds were more reliable cue to the parasitoids than that of volatiles emitted from the healthy plants. Le Ru and Makosso (2001) performed a similar experiment in a tritophic system involving cassava, mealybug and its coccinellid predator *E. flaviventris* Mader using a Y shaped olfactometer. The dual choice tests revealed that mealybug infested plants emit more volatile compared to the un-infested plants and attracted more predators.

Xie *et al.* (2004) carried out a study on a tritrophic system involving host plant bunge prickly ash, mealybug *Phenacoccus azaleae* (Kuwana) and its predator, *Harmonia axyridis* (Pallas) and they reported that herbivory resulted in an increased volatile emission which attracted the predators to the damaged host plant odors. GC-MS analysis revealed that new long-chain ester compounds were noted in the plant volatiles and also an increase in the quantity of certain compounds was recorded in the mealybug infested plants than that of healthy plants.

Gautam *et al*. (2010) extracted and evaluated the synomonal compounds from the healthy plant as well as *P. solenopsis* infested leaves of cotton genotype Sirsa P2 and the relative preference of the predator, *C. carnea* using a Y shaped olfactometer. The results revealed that *C. carnea* exhibited more preference towards the synomonal extracts of the mealybug infested leaves. The gas chromatographic analysis disclosed that a higher number of saturated

hydrocarbons up to eighteen compounds were present in the mealybug infested plant extracts compared to the healthy leaf extracts.

An investigation on the impact of various synomonal extracts on the behavior of *C. carnea* recorded that orientation response of the predator varied from extracts of different host plants such as tomato, brinjal and chilli. A six-arm olfactometer study revealed that mean orientation response was the highest in tomato and may be due to the difference in saturated hydrocarbon profile (Singh *et al.,* 2013). Halder *et al.* (2015) evaluated the impact of different hosts on mealybug *P. solenopsis* and its parasitoid *A. bambawalei* and the results revealed that tomato was the most preferred host for the parasitoid whereas pointed gourd was the least preferred one and substantiated the finding by suggesting the role of host-derived synomonal compounds on altering the efficiency of parasitoids.

2.8.2.3 *Role of Kairomones in Mediating Tritrophic Interactions among Mealybugs and Its Natural Enemies*

An experiment conducted to identify the orientation response of coccinellids *Exochomus* sp. and *Diomus* sp. on mealybugs *P. manihoti* and *P. citri* revealed that both predators were attracted to the wax and honeydew of the mealybugs. In a dual choice experiment, *Diomus* sp. exhibited a higher preference to the kairomonal compounds of *P. manihoti* than that of *P. citri.* Besides, wax of *P. manihoti* act as an arrestant for both coccinellids whereas honeydew attracted only *Exochomus* sp. (Meiracker *et al.,* 1990). Calatayud *et al.* (2001) reported that the parasitoids *Acerophagus coccois* Smith and *A. vexans* exhibited a preference towards the kairomone emitted from the body surface of mealy bug *Phenacoccus herreni* Cox & Williams as a cue to locate the host insect and also identified O-caffeoylserine as the compound by chromatographic methods.

Kotikal and Sengonca (1999) reported that both larvae and adults of *C. montrouzieri* exhibited a higher response towards olfactory cues emanated

from *P. citri* in an eight-arm olfactometer compared to the other host insects. The response of natural enemy, *B. suturalis* on the hexane body wash of *F. virgata* was evaluated and the results revealed that the highest response was elicited on the 0.75 g mL^{-1} concentration of kairomone. GC-MS analysis identified about thirteen compounds, of which octacosane was the major component followed by tricosane and n-heptacosane. Furthermore, field evaluation of kaolinite clay impregnated with kairomonal compounds sprayed on cotton plants attracted more number of *B. suturalis* (Singh, 2003). The volatile extracts of *P. solenopsis* attracted the predator *C. carnea* in a Y shaped olfactometer study signified the kairomonal activity of the mealybug body odors. The GC-MS studies identified a total of seventeen saturated hydrocarbons ranging from C_{13} to C_{30} in the kairomonal extract of mealybug (Gautam *et al.,* 2010).

Franco *et al.* (2008) evaluated the kairomonal response of the encyrtid, *Anagyrus* sp. to the sex pheromones, planococcyl acetate and (S)-(+)-lavandulyl senecioate of *P. citri* and *P. ficus* respectively. The female parasitoids showed a higher preference towards the sex pheromone of *P. ficus* in both field and olfactometer trials, which denoted the kairomonal role of (S)-(+)-lavandulyl senecioate. The variation in response of the parasitoid was based on its innate behavioral characters and also due to the intimate evolutionary relationship with *P. ficus*. Franco *et al.* (2009) suggested that the parasitoids used sex pheromones of host mealybugs as a kairomonal cue to efficiently locate the host, as it releases a strong volatile cue.

A study conducted in Italian vineyards to identify the impact of kairomone based attracting system on *Anagyrus* sp., a dominant parasitoid of the mealybug *P. ficus* disclosed that the vine mealybug sex pheromone (S)-(+)- lavandulyl senecioate act as a kairomone which increases the parasitization efficiency by acting as a chemical cue for the parasitoid (Mansour *et al.,* 2010). A field trial performed in the citrus orchards of Portugal, Italy and Israel, revealed that the per cent parasitization of *Anagyrus* sp. on *P. ficus* was significantly increased by the presence of lavandulyl senecioate (Franco *et al.,* 2011).

Tabata *et al.* (2011) identified cyclolavandulyl butyrate, a chemical compound used for the preparation of synthetic sex pheromone of *Planococcus kraunhiae* (Kuwana) as a kairomonal compound to the encyrtid parasitoid *Anagyrus sawadai* Ishii and suggested that the compound may be a part of the volatiles emitted from the body surface of mealybugs. Urbina *et al.* (2018) investigated on the response of *C. montrouzieri* to the sex pheromones of *Pseudococcus calceolariae* (Maskell) and *Pseudococcus viburni* (Signoret) in an olfactometer study. Both male and female predators exhibited a positive response towards the mealybug compounds though predators were more active towards *P. calceolariae* sex pheromone. The differential preference was substantiated by highlighting the co-evolutionary development of predator and *P. calceolariae.*

The volatiles produced by bacteria in the honeydew of mealybugs *M. hirsutus* and *Nipaecoccus viridis* were tested for the kairomonal activity towards the endoparasitoid, *Anagyrus dactylopii* (Howard). A total of ten bacteria were recovered and identified from the honeydew of mealybugs by adopting 16S rRNA technique and the volatiles collected by headspace sampling were evaluated for the preference shown by the parasitoid in an olfactometer. The study testified that mated females exhibited a positive response to the five bacterial volatiles which signified the kairomonal activity of the compounds in the honeydew. GC-MS analysis identified about six compounds, of which limonen-6-ol, pivalate was recognized as the common component, which can be further used for the enhancement of parasitoid population in pest management programmes (Fand *et al.*, 2020).

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled “Mealybugs of the vegetable ecosystems and tritrophic interactions of brinjal mealybugs” was conducted at Department of Agricultural Entomology, College of Agriculture, Vellayani during the period 2017 - 2020. The objectives of the present study were to identify the mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala along with other pertinent data including host range, natural enemy fauna and associated ant species. Furthermore, the study focused on the molecular characterization of the collected mealybugs and also to elucidate the tritrophic interactions among the host plant brinjal, mealybug and its natural enemy. The details on material utilized and methods adopted for the conduct of the research work are presented below.

3.1. COLLECTION AND IDENTIFICATION OF MEALYBUGS IN SOLANACEOUS AND CUCURBITACEOUS VEGETABLES

3.1.1 Documentation of Mealybugs Infesting Solanaceous and Cucurbitaceous Vegetables in Kerala

Purposive sampling was conducted at different agro ecological units of Kerala during 2017 to 2020 and documented mealybugs infesting solanaceous and cucurbitaceous vegetables. Besides, mealybugs infesting other vegetable crops were also recorded from the study area. The mealybugs were collected along with the plant part and the adult females were carefully separated using a soft camel brush without causing any damage to the specimen. The specimens were labeled with the locality, date of collection, collector’s name and the host plant accordingly and stored in vials with 75 % ethanol for further taxonomic identification. The survey locations along with the GPS coordinates are depicted in Table 2 and a location map was created using Arc GIS software (Fig. 1).

Table 2. Locations surveyed for the collection of mealybugs infesting solanaceous and cucurbitaceous vegetables and their natural enemies

District	Locations	GPS coordinates
Thiruvanathapuram	Instructional Farm Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”
	Balaramapuram	N $8^0$25’14.01” E 77^0 2’25.68”
	Thiruvallom	N $8^0$24’14.87” E 76^0 59’28.23”
	Panagode	N $8^0$25’22.62” E 76^0 58’ 17.4252”
	Amabalathara	N 8°27'08.0" E 76°57'02.2"
	Karavaram	N 8°45'09.4" E 76°48'52.9"
	Kulathoor	N 8°32'19.8" E 76°53'07.6"
	Chenkal	N 8°22'23.2" E 77°06'02.1"
Kollam	FSRS, Sadanandapuram	N 8°58'53.9" E 76°48'39.5"
	Poovattor	N 9°03'22.9" E 76°45'02.0"
	Ummannoor	N 8°56'02.6" E 76°48'45.1"
	Paravoor	N 8°48'52.6" E 76°40'11.3"
	Perumkulam	N 9°02'32.4" E 76°45'16.9"
	Kadakkal	N 8°49'48.5" E 76°55'12.2"
Pathanamthitta	Ezhamkulam	N 9°09'10.4" E 76°46'17.6"
	Prakkanam	N 9^0 16’ 14.88684” E 76^0 44’30.62004”
	Enathu	N 9°05'28.4" E 76°45'16.3"
Alapuzha	CPCRI, Kayamkulam	N $9^0$8’51.02” E 76^0 30’50.82”
	Koickal chantha	N $9^0$11’5” E 76^0 33’20.7”
	ORARS, Onattukkara	N $9^0$10’33.46” E$76^0$30’59.41”
Kottayam	RARS, Kumarakom	N $9^0$37’39.64”

		E 76^0 25’53.2”
Idukki	CRS, Pampadumpara	N 9°47'56.0" E 77°09'41.5"
	Prakandam	N $9^0$47’51.79092” E 77^0 8’59.36748”
	Valiyathovala	N $9^0$48’8.45028” E 77^0 7’57.04824”
	Mannakkudi	N $9^0$47’31” E 77^0 8’1.58”
	Anchumukku	N $9^0$48’10.53504” E 77^0 7’35.46552”
	Munnar	N 10°05'25.7" E 77°03'15.9"
Ernakulum	KVK, Ernakulum	N 10°02'33.5" E 76°12'24.9"
Thrissur	COH, Vellanikkara	N 10^0 32’43.5” E 76^0 17’0.4”
	KVK, Thrissur	N 10°32'49.3" E 76°16'05.5"
Palakkad	RARS, Pattambi	N $10^0$48’40.12812” E 76^0 11’ 25.82916”
	Muthalamada	N 10°38'14.3" E 76°48'02.4"
Malappuram	KVK, Tavanur	N $10^0$51’12.36348” E $75^0$59’ 13.15032”
	Vattamkulam	N 10°47'24.6" E 76°01'54.2"
Kozhikode	Kavilumpara	N 11°42'13.2" E 75°47'16.1"
Wayanad	RARS, Ambalawayal	N 11°36'59.8" E 76°12'52.2"
	Manjappara	N 11^0 36’14.06196” E 76^0 12’35.45856”
	Aandoor	N 11^0 35’17.16828” E 76^0 1’32.21832”
Kannur	PRS, Panniyur	N 12^0 4’ 47.6202” E 75^0 23’41.84016”
Kasargod	RARS, Pilicode	N 12°12'09.7" E 75°09'53.4"
	COA, Padannakkad	N 12°11'41.9" E 75°11'17.4"

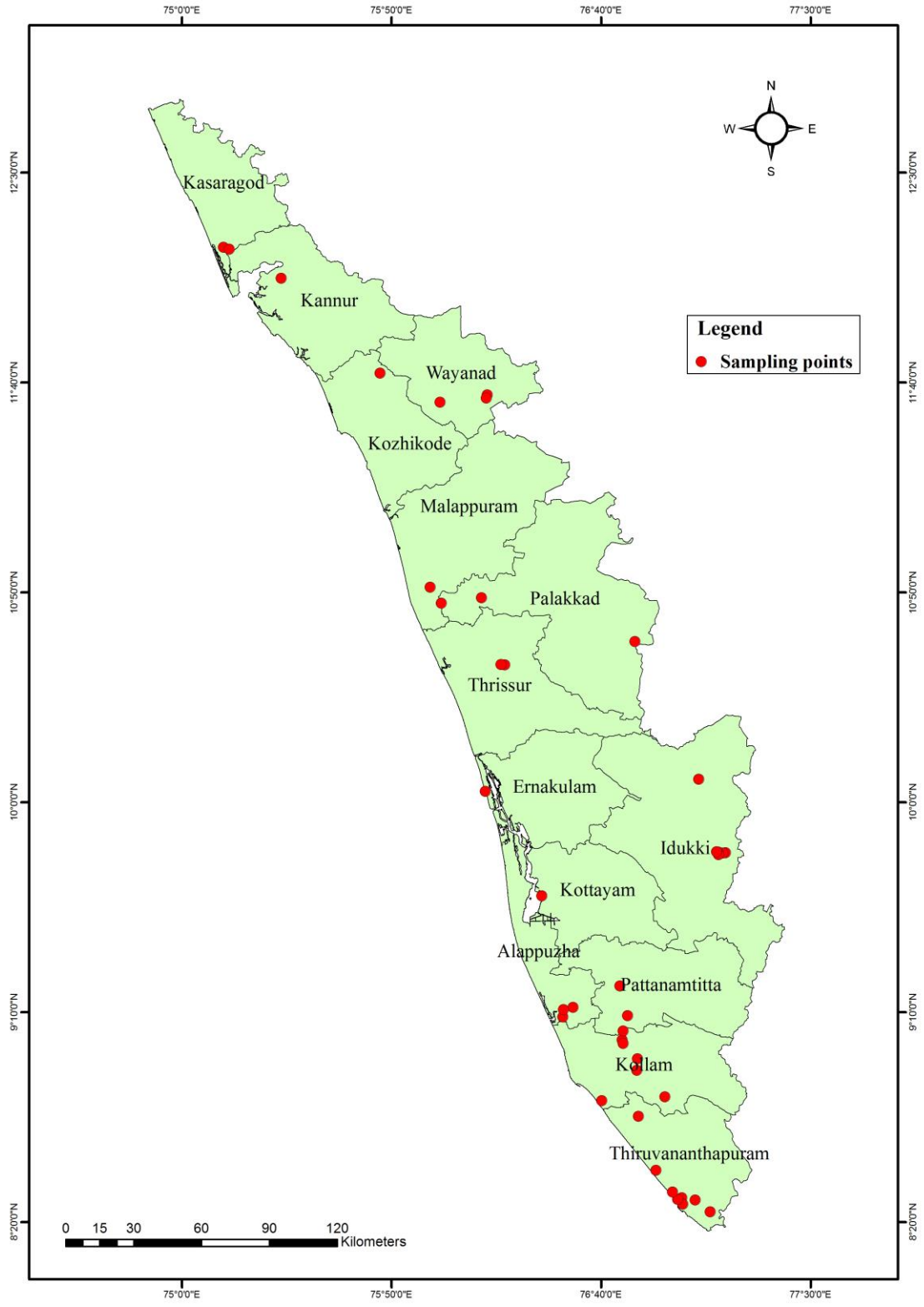


Fig. 1. Study area and locations surveyed for collection of mealybugs infesting solanaceous and cucurbitaceous crops in Kerala

3.1.2 Identification of Mealybug Species through Morphological Characterization

Morphological characters of slide-mounted specimens of adult females were used for taxonomic identification. The slides were prepared as per the procedure described by Sirisena (2013).

Materials required for the slide preparation are listed below;

- 10 % KOH (10g KOH dissolved in 100 mL distilled water)
- Acid Fuchsin stain (Acid Fuchsin stain powder- 0.5g, 10 % HCl- 25 mL and distilled water- 300 mL)
- Clove oil
- DPX mountant
- Distilled water
- 75 % ethanol, 95 % ethanol
- Micro spatula, needle, cavity blocks, watch glass, slides, coverslips and slide labels
- Dissection microscope

3.1.2.1 *Steps in Slide Preparation*

The steps involved in the preparation of slides include maceration, acidification, staining, dehydration, clearing, mounting and drying of the slides.

3.1.2.1.1 *Maceration*

The preliminary preservation in ethanol, dissolved the wax contents on the mealybug body to some extent. A small incision was made on the body of mealybug in between legs under a dissection microscope. This helps in faster penetration of the reagents and removal of the body contents. Later, the specimens were placed in 10 per cent KOH and heated at 60^{o} C in a heating block for 10 to 15 minutes until they turned translucent.

3.1.2.1.2 *Acidification*

The specimens were placed in distilled water in a cavity block for three minutes to remove the traces of KOH. The body contents were expelled out of the body using a spatula by gently tapping on the body surface. Thereafter the specimens were transferred to 80 % ethanol to acidify the cuticle.

3.1.2.1.3 *Staining*

The specimens were immersed in Acid Fuchsin stain for 15 to 30 minutes (time varied with the nature of the specimens) until the tissues absorbed the sufficient quantity of stain. After staining, the specimens were rinsed for a while in distilled water to remove the excess stain.

3.1.2.1.4 *Dehydration*

The specimens were transferred to 75 % ethanol for 1 to 2 minutes followed by immersion in 95 % ethanol for about 15 minutes. The excess of stain adhering to specimen was removed in this step along with the replacement of water inside the cuticle with ethanol for the better fixing of the stain.

3.1.2.1.5 *Clearing*

The dehydrated specimens were transferred to anhydrous clove oil to remove the residual fat and oil globules in the specimens. The specimens were soaked in clove oil for about 10 minutes and egg shells or any other remnants were carefully removed from the mealybug body using a micro needle.

3.1.2.1.6 *Mounting*

The specimen was placed on the centre of a good quality microscopic slide over a drop of clove oil in such a way that the head pointing towards the person handling the microscope. After positioning the body parts, excess clove oil was removed from the slide and added with a drop of DPX into the slide. A coverslip was slowly placed over it using a needle without imparting any force on the coverslip. The slides were labeled with necessary details such as the place of

collection, host plant information, date of collection and the name of the collector to avoid future confusions during the time of identification.

3.1.2.1.7 ***Drying of the Slides***

The slides were kept in an incubator at 40^0C for 2 to 3 month for proper drying.

3.1.2.2 ***Photomicrography and Morphometrics***

The slides were observed under a compound microscope (Carl Zeiss with 5X, 10X, 40X and 100X objectives) and the important characters were photographed using a Canon 1100 D camera using EOS utility software and the images were stacked using Helicon focus 6 software. Whole size photograph of slide-mounted specimens was taken in a stereomicroscope (Carl Zeiss Stemi 508).

The morphological characters of mealybug with taxonomic importance is depicted in Plate 1.The measurements of five slide-mounted mealybug specimens were taken using an ocular micrometer placed inside the compound microscope. The measurements taken were given below.

1. Total length measured as the distance from the anterior side of the body to the posterior end (µm)
2. Total breadth measured as the total width of the body at the widest portion (µm)
3. Length of the antenna from base to the tip (µm)
4. Length of hind tibia (µm)
5. Length of hind tarsus (µm)
6. Length of hind trochanter (µm)
7 .Length of hind femur (µm)
8. Length of hind tibia + tarsus (µm)

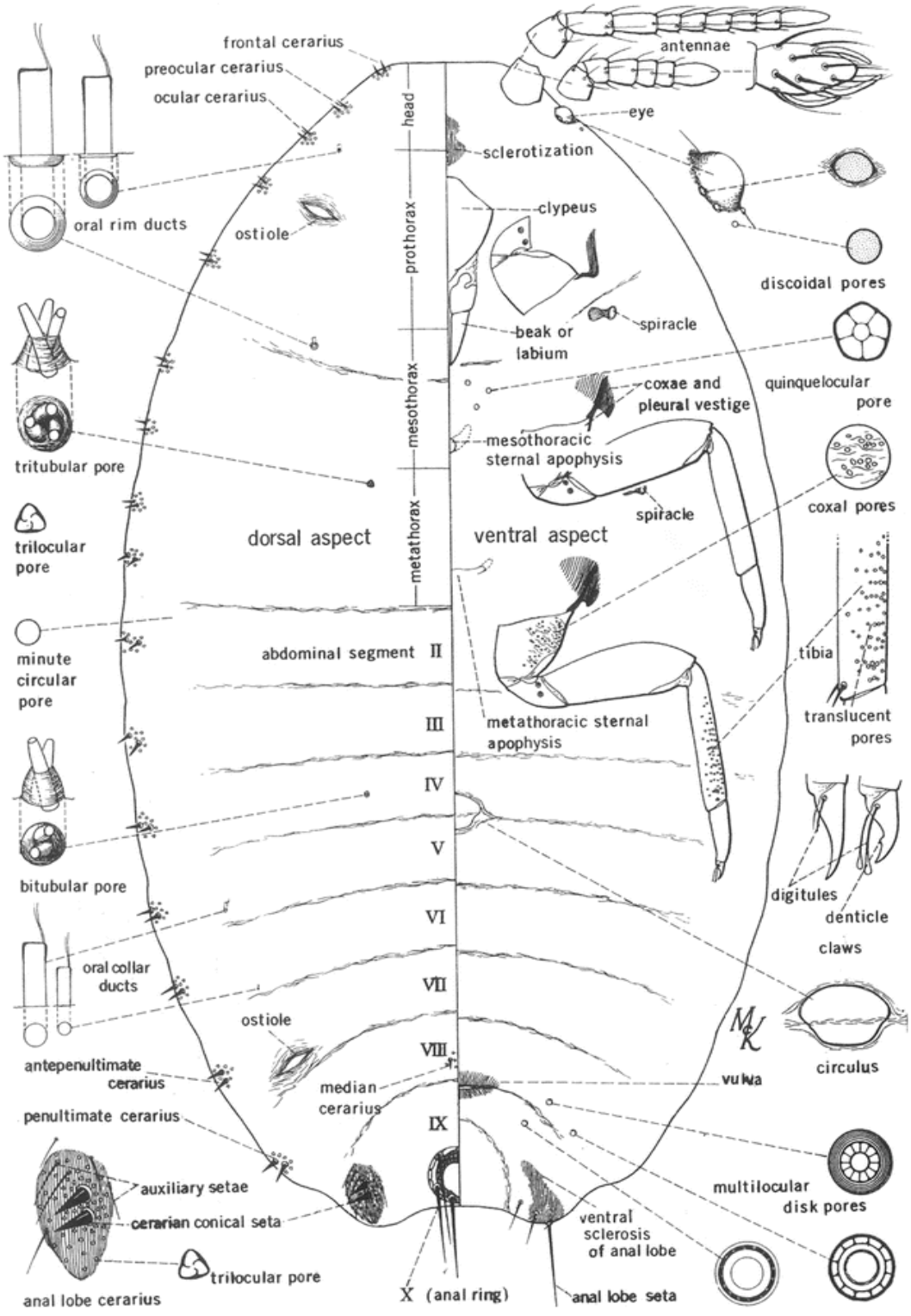


Plate 1: General morphology of an adult female mealybug (Source: Williams, 2004).

9. Length of hind trochanter +femur (µm)

10. Ratio of length of hind tibia+ tarsus to length of hind trochanter + femur

11. Ratio of hind tibia to tarsus

12. Total width of anal ring (µm)

13. Length of anal ring setae (µm)

14. Length of dorsal setae (µm)

15. Length of ventral setae (µm)

16. Length of oral collar tubular duct (µm)

17. Width of multilocular pore (µm)

18. Width of quinquelocular pore (µm)

19. Width of trilocular pore (µm)

3.1.2.3 *Identification of Mealybugs*

The slide-mounted specimens were identified using the taxonomic key provided by Williams (2004). The mealybug specimens were sent to Dr. Sunil Joshi, Division of Insect Systematics, ICAR- National Bureau of Agricultural Insect Resources, Bangalore for species-level confirmation. The slides were deposited in the Travancore insect collection of the Department of Agricultural Entomology at College of Agriculture, Vellayani.

3.1.3 Distribution of Mealybug Species in Kerala

The distribution map of mealy bug species collected from Kerala was created using the ArcGIS software. The details on GPS locations and species data were given in Annexure I.

3.1.4 Study of Morphological and Molecular variations in Brinjal Mealybug *C. insolita*

3.1.4.1 *Morphological Variations*

Brinjal mealybug, *C. insolita* was collected from different parts of Kerala to study the morphological variations. The observations were made using five slide-mounted adult female specimens representing each location. A total of 19 characters as mentioned in 3.1.2 were measured under an ocular micrometer in a compound microscope and the data were analyzed to verify the morphological variations exhibited by the mealybugs. Subsequently, the data were subjected to multivariate analysis (Principal Component Analysis - PCA and Canonical Discriminant Analysis - CDA) with the statistically significant characters to identify the differences between the mealybugs collected from different parts of Kerala.

3.1.4.2 *Molecular Variations*

The mealybug populations exhibiting morphological variations were subjected to molecular characterization. The steps involved in molecular characterization of mealybugs are given below.

3.1.4.2.1 *DNA Isolation from Mealybug*

The mealybug specimens were collected from different parts of Kerala and preserved in absolute alcohol at $^{-}80^{0}$ C in a low-temperature cabinet. The genomic DNA of mealybug was isolated using QIAGEN DNeasy® blood and tissue kit. A single specimen of mealybug was used for the DNA extraction and ground using liquid nitrogen and placed inside a microcentrifuge tube. The DNA extraction procedure was as per the guidelines provided by the manufacturer.

3.1.4.2.2 *Polymerase Chain Reaction*

The isolated DNA was amplified using a primer specific to the mitochondrial cytochrome oxidase 1 subunit region 2. The primers selected for the PCR reaction were COI-J-2183-F-CAACATTTATTTTGATTTTTTGG and

COI-N-2568-R-GCWACWACRTAATAKGTA TCATG (Gullan and Martin, 2003). The PCR reaction was performed in a thermal cycler using 25µl volume of reaction mixture containing 1 µl of template DNA, 1 µl forward primer, 1 µl reverse primer, 12.5 µl of PCR master mix and 9.5 µl molecular water. The PCR conditions were programmed as; initial denaturation at 94 ^{0}C for 4 minutes followed by 35 cycles of denaturation at 94 ^{0}C for 30 seconds, annealing at a temperature of 45 ^{0}C for 60 seconds and extension at 72 ^{0}C for 2 minutes and a final extension at 72 ^{0}C for 10 minutes and stored at 4^{0}C. Agarose gel (1%) electrophoresis was carried out to confirm the presence of DNA and the amplicon was sent to Rajiv Gandhi Center for Biotechnology (RGCB), Thiruvananthapuram for nucleotide sequencing. The nucleotide sequences were checked for sequence similarity using nucleotide BLAST (Basic Local Alignment Search Tool) at National Center for Biotechnology Information (NCBI) database.

3.1.5 Documentation of Host Range of Mealybugs Infesting Solanceous and Cucurbitaceous vegetables

The host range of mealybugs infesting solanaceous and cucurbitaceous crops were recorded from different regions of Kerala and was identified and confirmed with experts from concerned disciplines. The host plants of each mealybug was separately documented and categorized as vegetables, fruits, tubers, ornamentals, spices, medicinal plants, fibers, oilseeds, pulses, beverages and weeds. The level of infestation of mealybugs on different host plants was recorded as per the scales developed by Arif *et al*. (2009).

Parameters	**Level of infestation**
Incidental found (I)	Relatively low number of individuals noticed in the plant. No signs on the breeding of population
Low (L)	Mealybugs of all stages were recorded in the host plant without any notable damage symptoms on the plant

Medium (M)	Mealybugs of all stages were observed in large numbers. Yellowing and wilting symptoms on plant, but not causing the death of plant.
High (H)	Mealybugs of all stages were observed in large numbers. Most of the plant parts were covered with mealybugs leads to the death of the plants.

3.1.6 Documentation of Natural Enemies of Mealybugs

3.1.6.1 *Predators*

The coccinellid predators associated with the mealybugs were collected and killed using ethyl acetate. The specimens were preserved in 75 % ethanol and brought to the laboratory. The specimens were mounted on triangular card pieces that were held in a unit tray using an insect pin of size 2. The beetles were glued to the tip of the paper in such a way that the right side of thorax touches the paper strip. The specimens were labeled with details *viz.*, host insect, date of collection, name of the collector, locality and the host plant. The specimens were dried in a hot air oven at 50^0C for 5 days and later transferred into an airtight insect box. The coccinellid grubs predating on mealybugs were collected along with the plant part and brought to the laboratory for rearing. The emerged beetles were killed and preserved as per the aforementioned procedures and sent for identification.

The maggots of midges feeding on mealybugs were collected and reared in the laboratory. The emerged adults were preserved in 75 % ethanol and sent for identification. The specimens were also mounted on triangular card pieces that were held in a unit tray using an insect pin of size 2. The specimens were labeled with details *viz.*, host insect, date of collection, name of the collector, locality and the host plant. The specimens were dried in a hot air oven at 50^0C for 5 days and later transferred into an airtight insect box and sent for identification.

The immature stages of drosophilid predators were also collected and reared in the laboratory. The emerged specimens were preserved in 75 % ethanol and sent for identification. The specimens were also mounted on triangular card

pieces that were held in a unit tray using an insect pin of size 2. The specimens were labeled with details *viz.,* host insect, date of collection, name of the collector, locality and the host plant. The specimens were dried in a hot air oven at 50^0C for 5 days and later transferred into an airtight insect box and sent for identification.

The immature stages of chrysopid predators were collected from the field and reared in the laboratory. The emerged adult specimens were preserved in 75 % ethanol. Some of the specimens were pinned using an insect pin of size 3, after extending the wings, dried and kept in an insect box and sent for identification.

3.1.6.2 *Parasitoids*

Parasitized mealybugs from the field were collected and kept for adult emergence in plastic containers. The emerged parasitoids were preserved in 75 % ethanol and were identified. The specimens were also mounted on triangular card pieces that were held in a unit tray using an insect pin of size 2. The specimens were labeled with details *viz.,* host insect, date of collection, name of the collector, locality and the host plant. The specimens were dried in a hot air oven at 50^0C for 5 days and later transferred into an airtight insect box

3.1.7 Documentation of Ants Associated with Mealybugs

The ant species associated with mealybugs were collected and preserved in 75 % ethanol. The specimens were also mounted on triangular card pieces that were held in a unit tray using an insect pin of size 2. The specimens were labeled with details *viz.,* host insect, date of collection, name of the collector, locality and the host plant. The specimens were dried in a hot air oven at 50^0C for 5 days and later transferred into an airtight insect box. The specimens were identified up to the genus level using the taxonomic key published by Bolten (1994). Later the specimens were sent for species level confirmation.

3.1.8 Maintenance of Mealybug Culture

Mealybugs were reared on pumpkin fruits, potato sprouts and also on susceptible host plants in the laboratory of the Department of Agricultural

Entomology, College of Agriculture, Vellayani for carrying out experiments on taxonomic identification, rearing of natural enemies, molecular characterization and tri- trophic interaction studies.

3.1.8.1 *Mass Rearing of Mealybugs on Pumpkin*

Ripe, good quality medium-sized pumpkin with ridges and grooves were selected for the rearing of mealybugs. The select pumpkins were properly washed to remove the adhering dirt particles and treated with 0.1 per cent carbendazim to prevent the fungal infection. Damaged surface portions were covered with melted paraffin wax, shade dried and kept inside an insect rearing cage. A coir rope was tied over the fruit surface to promote the better establishment of the mealybug population. Ovisacs/nymphs of mealybugs collected from different locations/crops were carefully transferred to these pumpkin for the population build-up (Plate 2) (Padmanabhan, 2017).

3.1.8.2 *Mass Rearing of Mealybugs on Potato Sprouts*

Healthy, medium-sized potatoes were selected and washed thoroughly in water to remove the adhering dirt particles from the surface. Sterilized sand was filled in a tray and potatoes were placed and kept inside a rearing cage. When the sprouts reached a height of 3 to 4 inches, adult mealybugs (*C. insolita*) were inoculated to the sprouts. It was kept at a temperature of 27^0C and 60 % humidity for population buildup (Plate 3) (Padmanabhan, 2017).

3.1.8.3 *Mass Rearing of Mealybugs on Host Plants*

The mealybug species that were reluctant to establish on pumpkin and potato sprouts were maintained in the susceptible host plant itself. Healthy disease-free seedlings were transplanted in small pots and placed inside an insect rearing cage. Nymphs and adults of mealybugs were inoculated into the plant using a soft camel brush or mealybug infested twigs were attached to the host plants using a cello tape or leaf bits containing mealybugs were stapled on the undersurface of leaves. Additional care was given to remove the parasitized

Plate 2

Plate 3

Plate 4

Plate 5

Plate 2. Mass rearing of mealybug, *Planococcus* sp. on pumpkin, Plate 3. Mass rearing of mealybug, *Coccidohystrix insolita* in potato, Plate 4. Mass rearing of mealybug, *C .insolita* in host plants, Plate 5. Field experiment

mealybugs and immature stages of predators from the twig and leaf bits under a stereomicroscope (Plate 4).

3.2 MOLECULAR CHARACTERIZATION OF MEALYBUGS COLLECTED FROM BRINJAL AND PUMPKIN

3.2.1 Isolation of Genomic DNA, Polymerase Chain Reaction and Sequencing

The mealybug specimens collected from different parts of Kerala were preserved in absolute alcohol and stored in -80^{0}C in low temperature cabinet. The molecular sequencing of the specimens were carried out as per *3.1.4.2.1.* and *3.1.4.2.2.*

3.2.2 Sequences Analysis and Submission of Sequence to NCBI GenBank and *BOLD (*Barcode *of Life Data System)*

The nucleotide sequences obtained were checked for sequence similarity using nucleotide BLAST (Basic Local Alignment Search Tool) at National Center for Biotechnology Information (NCBI) database. The annotated nucleotide sequences were submitted to NCBI GenBank. The sequences were also submitted to BOLD along with specimen details, primer details and images and generated DNA barcode for each specimen (Mathew, 2015).

3.3 MOLECULAR CHARACTERIZATION OF ENDOSYMBIONTS OF *C. INSOLITA.*

The fresh samples of mealybug *C. insolita* was collected and the metagenomic DNA was isolated from the specimen. The molecular characterization of endosymbionts of mealybug, *C. insolita* was carried out at Eurofins Genomics India Pvt. Ltd, Bengaluru and the steps are given below.

3.3.1 Isolation of Metagenomic DNA from *C. insolita*

Mealybugs were surface sterilized in sodium hypochlorite (0.1%) for 30 sec and in ethanol (70%) for 30 sec to remove the adhering contaminants. The samples were transferred to microcentrifuge tubes and added with 60^{0}C pre-

warmed CTAB (600 µl) +3 µl of Beta-Mercaptoethanol+ 10 µl of 20 % SDS and ground using a micropestle. 3 µl of Proteinase-K was added to the tube and vortexed for 5 minutes. The extract was transferred to a centrifuge tube and kept for overnight incubation. After incubation, samples were cooled to room temperature and centrifuged at 14,000 rpm for 10 minutes. The supernatant was taken and an equal volume of Phenol: chloroform: Isoamyl alcohol (25:24:1) was added to it and vortexed for 5 minutes. It was followed by centrifugation at 14000 rpm for 10 minutes and the supernatant was collected and mixed with 600 µl of chloroform: isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 10 minutes. 400 µl of chilled isopropanol was added to the supernatant and kept at -35 or-50^0C in deep freezer for 1h. The samples were centrifuged at 10000 rpm for 10 minutes and the supernatant was decanted and added with 400 µl 70 % chilled ethanol + 100 µl ammonium acetate to the pellet for washing. It was followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant was carefully removed and added with 400 µl of absolute alcohol and centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet was dried at room temperature and dissolved in nuclease free water for storage. The quality and quantity of DNA was checked using Nanodrop by determining the A260/280 ratio.

3.3.2 Polymerase Chain Reaction with Metagenomic DNA

The PCR reactions were carried out using the isolated DNA along with the bacterial 16S V3-V4 region specific primer set. The primers used were 16S rDNA F-GCCTACGGGNGGCWGCAG and 16S rDNA R-ACTACHVGGGTATCTAATCC.

The PCR reactions were carried out with 5µl PCR buffer, 2 µl dNTP mix (200µM), 1 µl forward primer, 1 µl reverse primer, 0.35 µl Taq polymerase 2µl template DNA and made up to 50 µl with nuclease free water. The PCR conditions were initial denaturation at 94^0C for 1 minute, denaturation at 94^0C for 1 minute, primer annealing 54^0C for 1 minute and primer extension at 72^0C for

1.5 minute followed by extension at 72^0C for 7 minutes and stored at 4^0C. The PCR product was resolved on 1.2 % Agarose gel at 120 V for 60 minutes.

3.3.3 16S rRNA Library Preparation and Sample Loading

The metagenomic DNA of mealybug, *C. insolita* was taken and standard protocol was followed for 16S rRNA library preparation and the sample loaded to the Illumina MiSeqTM sequencer.

3.3.4 Illumina Sequencing Data and Bioinformatics Analysis

The raw paired end sequencing reads obtained from Illumina MiSeq sequencer were checked for quality parameters. High quality clean reads were obtained using Trimmomatic v0.38 by removing adapter sequences, ambiguous reads (reads with unknown nucleotides "N" larger than 5%), and low-quality sequences (reads with more than 10% quality threshold (QV) < 20 phred score). High quality reads were joined together and operational taxonomic units (OTU) were selected based on sequence similarity within the reads using UCLUST program at 97 % sequence similarity. A representative sequence of 16S bacteria from each OTU was selected against Greengene database (version 13_8) and the taxonomic identity was confirmed. Diversity metrics of the endosymbionts were calculated and compared the types of communities, using the taxonomic assignments and pie diagrams were constructed.

3.4 TRI-TROPHIC INTERACTION

A set of experiments were conducted to study the tri-trophic interaction among different brinjal cultivars, mealybug *C. insolita* and its natural enemies in the laboratory using olfactometer and also in the field.

3.4.1 Interaction in Brinjal Ecosystem Mediated by Mealybug *C. insolita* and Its Natural Enemies.

A field experiment was conducted at the Instructional Farm, Vellayani in a randomized block design with three replications from November 2018 to May 2019. A total of ten brinjal cultivars were selected and screened for their response

towards mealybug, *C. insolita* and its natural enemies (Plate 5). The selected brinjal cultivars include KAU/ICAR released varieties and hybrids (Table 3).

Thirty days old seedlings were transplanted in the main field with a spacing of 60 x 60 cm with 10 plants per replication. All the agronomic practices recommended by Kerala Agricultural University (KAU, 2016) were adopted for raising the crop except the plant protection measures. Observations on the infestation of mealybugs in different brinjal cultivars along with the natural enemies were recorded from the experimental plots.

3.4.1.1 *Estimation of Mealybug Population in Brinjal Cultivars*

Observations were taken from five randomly selected plants from each plot at ten days intervals starting from two months after transplanting. From each plant, three leaves were selected randomly from the top, middle and bottom portions and were tagged for counting the population of both nymphs and adults of mealybugs by adopting the window method (Padmanabhan, 2017). The average population of mealybugs in five observational plants were calculated and represented as the mean number of mealybugs/ three leaves.

3.4.1.2 *Assessment of Leaf Damage Caused by Mealybug*

The per cent leaf damage was calculated by dividing the number of leaves infested by mealybugs with the total number of leaves present in the plant and multiplying with 100. Per cent leaf damage of five observational plants from each plot were taken and average was calculated and denoted as mean per cent leaf damage.

Table 3. Details of brinjal cultivars selected for the study

Sl. No	Cultivar	Source
1	Haritha	Department of Vegetable Science, COA, Vellayani
2	Neelima	Department of Vegetable Science, COA, Vellayani
3	Ponni	Department of Vegetable Science, COA, Vellayani
4	Pink Long	Private seed Company
5	Udit	Private seed Company
6	Green Long	Private seed Company
7	Pusa Purple Long	IARI, New Delhi
8	Pusa Kausal	IARI, New Delhi
9	Pusa Uttam	IARI, New Delhi
10	Pusa Shymala	IARI, New Delhi

3.4.1.3 *Categorization of Cultivars Based on Leaf Damage*

The brinjal cultivars were categorized into different groups based on the leaf damage caused by *C. insolita*. A modified tolerance index was developed based on Elanchezhyan *et al.* (2008).

Leaf damage (%)	Ratings	Category
0	0	Immune/Highly resistant
1-10	1	Resistant
11-20	2	Moderately resistant
21-30	3	Moderately susceptible
31-40	4	Susceptible
>41	5	Highly susceptible

3.4.1.4 *Estimation of Natural Enemy Population*

The population of natural enemies were taken from five randomly selected plants from each plot and represented as mean population of natural enemies per plant.

3.4.2 Biophysical Parameters Mediating Tri-trophic Interaction in Brinjal

3.4.2.1 *Trichome Density*

The third fully opened leaf from three randomly selected plants of each cultivar was collected for the estimation of trichome density. Leaf bits of size $1cm^2$ were taken and the number of trichomes were counted using a stereomicroscope and represented trichome density as number of trichomes per cm^2.

3.4.2.2 *Leaf Thickness*

Leaves of similar growth stage were excised from three randomly selected plants of each cultivar. Leaf thickness was calculated by taking the cross-section

of the leaf lamina and measured using an ocular micrometer under a stereomicroscope and expressed in mm.

3.4.2.3 *Length Width Ratio of Leaf*

Length width ratio of the leaf was calculated by dividing the total length of the leaf to the total width of the leaf at the broadest region.

3.4.2.4 *Number of Branches*

The total number of branches in the plant was recorded.

3.4.2.5 *Plant Height*

The height of the plant was measured from base to the topmost leaf bud using a measuring scale and expressed in cm.

3.4.2.6 *Biophysical Factors of Brinjal Cultivars with Mean Population of Mealybug and Natural Enemies*

The mean population of mealybugs and natural enemies were correlated with biophysical parameters of brinjal cultivars and the correlation coefficients were recorded. A multiple regression analysis was carried out and regression equations on the mean population of mealybugs and mean population of natural enemies were developed based on biophysical characters.

3.4.3 Biochemical Parameters Mediating Tri-trophic Interaction in Brinjal

3.4.3.1 *Total Phenol Content*

The total phenol content of the leaf was estimated by the Folin - Ciocalteau reagent method as suggested by Malick and Singh (1980). Leaf sample (0.5 g) was ground on a pestle and mortar using 5 to 10 mL of 80 % ethanol. The sample was centrifuged at 10000 rpm for about 20 minutes and the supernatant was collected in a test tube. The residue was re-extracted using 80 % ethanol and the supernatants were pooled together. It was kept in a water bath to evaporate up to dryness and the leftover residue was dissolved in 5 mL of distilled water. 0.5 mL aliquot was pipetted out into a fresh test tube. After the volume was made up

to 3 mL using distilled water, 0.5 mL Folin - Ciocalteau reagent was added to the test tube and kept it for 3 minutes. Na_2CO_3 20 % (2 mL) was added to each test tube and placed in boiling water for exactly one minute. After cooling, the absorbance was read at 650 nm in a spectrophotometer.

A standard curve was prepared using different concentrations of catechol. Based on the standard curve, the concentration of phenol in the test samples were calculated and expressed in mg g^{-1} fresh weight.

3.4.3.2 ***Total Protein Content***

The total soluble protein content of leaf samples was estimated as per the method described by Bradford (1976). Leaf sample of 0.1 g was ground in a pestle and mortar by using 10 mL of phosphate buffer (pH 7.8). This was centrifuged at 5000 rpm for about 10 minutes and the supernatant was collected for the protein estimation. From this, 20 µl aliquot was taken in a test tube and added 5 mL of Bradford dye-binding solution. The solution was mixed properly and kept for 5 minutes for the blue color development. The absorbance was read at 596 nm.

A standard curve was formulated by using different concentrations of bovine serum albumin (BSA). The concentration of protein in test samples were calculated from the standard curve and expressed as mg g^{-1} fresh weight.

3.4.3.3 ***Total Reducing Sugar***

The total reducing sugar of leaf samples were estimated by Dinitrosalicylic Acid method as described by Miller (1972). Weighed out 0.1 g of leaf sample and was ground in a pestle and mortar using 5 mL of hot 80 % ethanol. This step was repeated with another 5 mL of hot 80 % ethanol and the extract was centrifuged. The supernatant was collected and kept in a water bath at a temperature of 80°C to evaporate the contents up to dryness. The leftover residue was dissolved in approximately 10 mL of water and 0.3 mL aliquot was transferred into a test tube. The volume was made up to 3 mL using water and added 3 mL of DNS reagent in each test tube and kept in a water bath for 5 minutes. 1 mL of 40 per cent

Rochelle salt solution was added to the test tube and the absorbance was read at 510 nm.

A standard curve was formulated by using different concentrations of glucose. The reducing sugar content of the test samples was calculated from the standard curve and represented as mg g^{-1} fresh weight.

3.4.3.4 ***Total Chlorophyll Content***

The total chlorophyll content of the leaf was estimated by using the procedures suggested by Hiscox and Israelstam (1979). Leaf sample (0.5 g) was taken and cut it into small pieces and placed in a test tube containing 10 mL of DMSO: 80 % acetone mixture (1:1 v/v). The solution was kept overnight at room temperature. The solution was made up to 25 mL using DMSO - acetone mixture and the absorbance was read at 663, 645, 480 and 510 nm in a spectrophotometer. The amount of chlorophyll in leaf samples was calculated using the following equation and represented as mg g^{-1} fresh weight.

$$Total\ Chl\ (a+b) = (8.02 \times A_{663} - 20.2 \times A_{645}) \times \frac{V}{1000} \times \frac{1}{fresh\ weight}$$

3.4.3.5 ***Total Carotenoid Content***

The total carotenoid content of the leaf was estimated by using the procedures suggested by Hiscox and Israelstam (1979). Leaf sample (0.5 g) was taken and placed in a test tube containing 10 mL of DMSO: 80 % acetone mixture (1:1 v/v). The solution was kept overnight at room temperature. The solution was made up to 25 mL using DMSO - acetone mixture and the absorbance was read at 480 nm and 510 nm in a spectrophotometer. The amount of carotenoids in leaf samples were calculated using the following equation and represented as mg g^{-1} fresh weight.

$$\text{Total carotenoid content} = \{(7.6 \times A480) - (1.49 \times A510)\} \times V/1000 \times W$$

3.4.3.6 *Biochemical parameters of Brinjal Cultivars with Mean Population of Mealybug and Natural Enemies*

The mean population of mealybugs and natural enemies were correlated with biochemical parameters of brinjal cultivars and the correlation coefficients were recorded. A multiple regression analysis was carried out and regression equations on the mean population of mealybugs and mean population of natural enemies were developed based on biochemical characters.

3.4.4 Info- Chemical Mediated Tri-trophic Interaction in Brinjal, Mealybug and Natural Enemies

The tri-trophic interactions between brinjal cultivars, mealybug *C. insolita* and its natural enemies were studied based on the info-chemicals released from the plants and mealybugs.

3.4.4.1 *Extraction of Synomones from Uninfested Brinjal*

Ten different cultivars of brinjal were maintained in insect -proof nylon cage separately inside the net house (Plate 6). The synomonal compounds were extracted from healthy leaf samples as per the procedure given below.

Leaf sample (10g) was taken from each plant and immersed overnight in 100 mL of HPLC grade distilled hexane in glass bottles. The hexane extract was filtered through a Whatman No. 1 filter paper and anhydrous sodium sulphate (1g) was added and kept for 2h for dehydration. The hexane extract was subjected to column chromatography and was passed through the silica gel of 60 to 120 mesh size. The eluted compound was collected and distilled at a temperature of 60 to 70^0 C in a rotary vacuum flash evaporator. The leftover residue was collected by rinsing the flask with HPLC grade hexane in a small glass vial. The compounds were stored at $^-80$ ^{0}C in a low-temperature cabinet (Trang, 2008).

Plate 6. Insect proof cage inside the net house for maintaining plants for synomone extraction

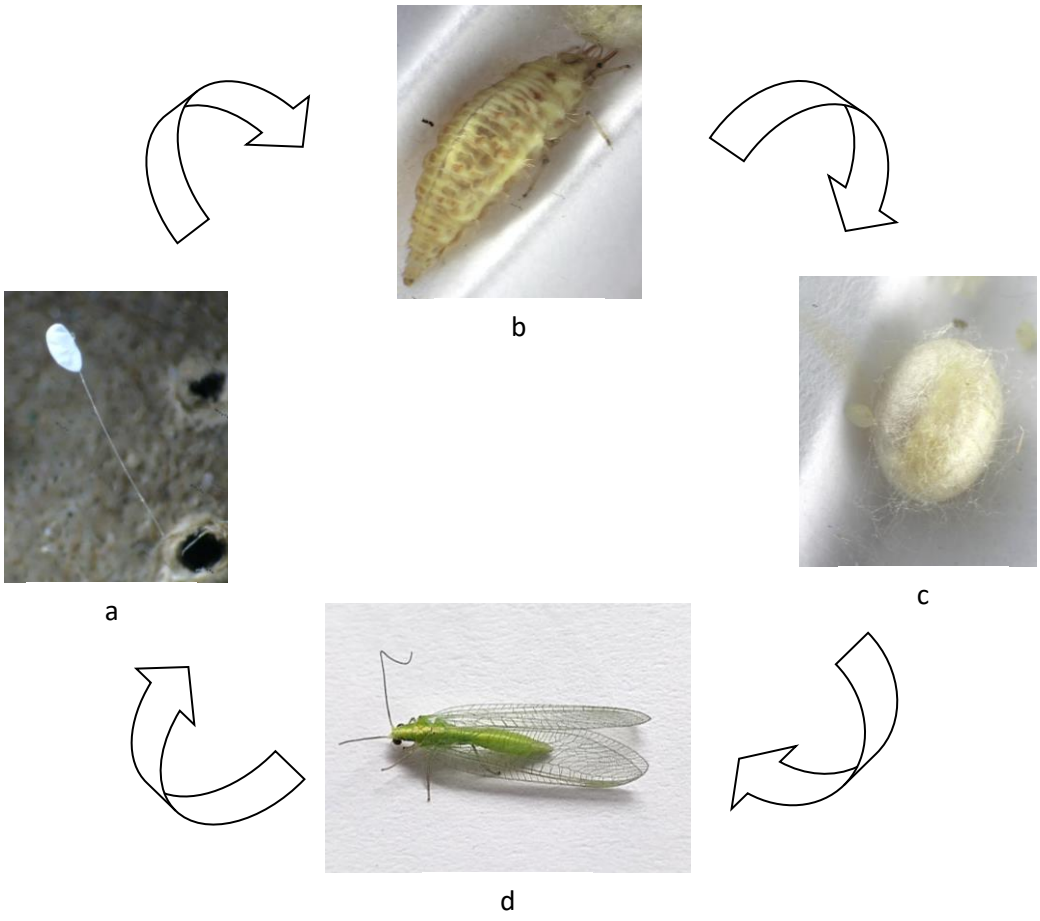


Plate 7. Life stages of the predator *C. zastrowii sillemi*

a. egg, b. larva, c. pupa, d. adult

3.4.4.2 *Extraction of Synomones from Brinjal Infested with the Mealybug C. insolita.*

Ten different cultivars of brinjal were maintained under an insect-proof nylon cage in a net house. Twenty adult mealybugs were carefully inoculated into each plant using a soft camel brush at one month after transplanting.

About twenty days after inoculation, the synomonal compounds were extracted from the mealybug infested leaf samples as per the procedure mentioned in 3.4.4.1

3.4.4.3 *Maintenance of Natural Enemy Culture*

A nucleus culture (eggs) of the predator, *Chrysoperla zastrowi sillemi* Esben-Peterson was purchased from NBAIR, Bangalore and reared in the laboratory. The eggs (100 nos.) (Plate 7a) were mixed with 0.75 cc of sterilized eggs of *Corcyra cephalonica* Stainton in a plastic container. The emerged larvae (Plate 7b) were transferred separately in to small plastic boxes and reared on the diet containing eggs of *C. cephalonica*. Total quantity of *Corcyra* eggs required for rearing 100 chrysopid larvae was 4.25 cc. Brown paper was provided for facilitating coccon (Plate 7c) formation in the plastic boxes. The coccons were collected and placed in a glass jar for adult emergence. The emerged adults (Plate 7d) were maintained with a diet containing 50 % honey and castor pollen. The adult chrysopids were used for further studies.

3.4.4.4 *Response of C. zastrowi sillemi to Synomonal Compounds of Brinjal*

3.4.4.4.1 *Multi-armed Olfactometer Assay*

Relative response of the predator *C. zastrowi sillemi* towards the synomonal compounds of various brinjal cultivars was evaluated in a multi-armed olfactometer. The olfactometer made up of glass with a central portion of 15 cm diameter and arm length of 10 cm and diameter of 2.5 cm was used for the study (Plate 8). The olfactometer was kept at a temperature of 27 ^{0}C and relative

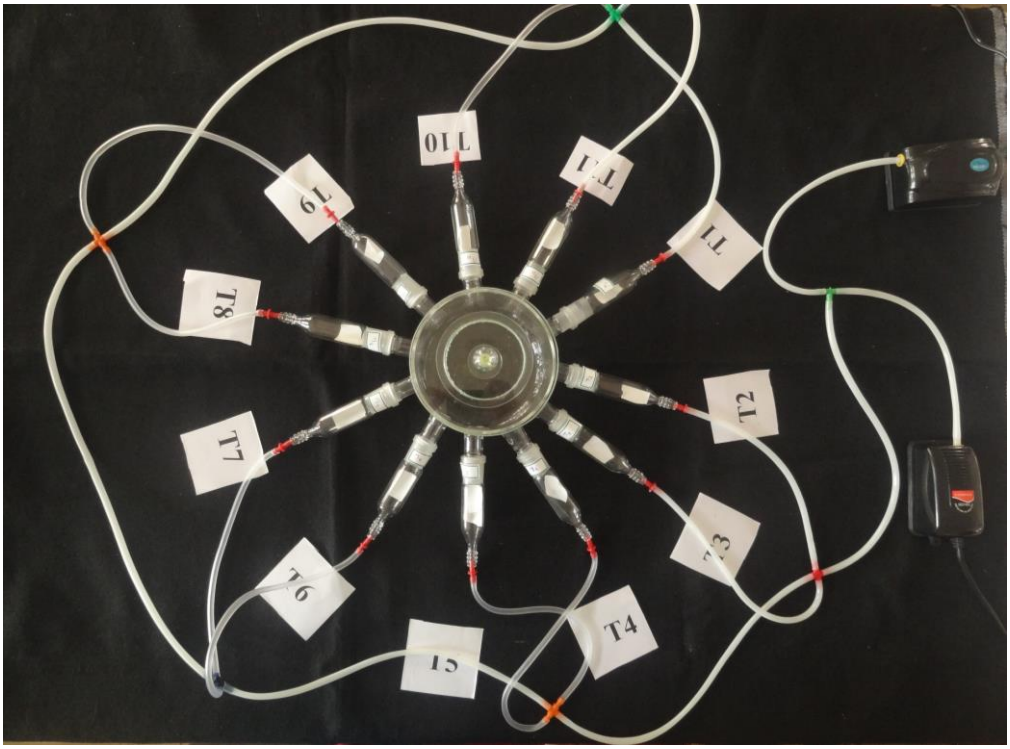


Plate 8. Multi armed olfactometer

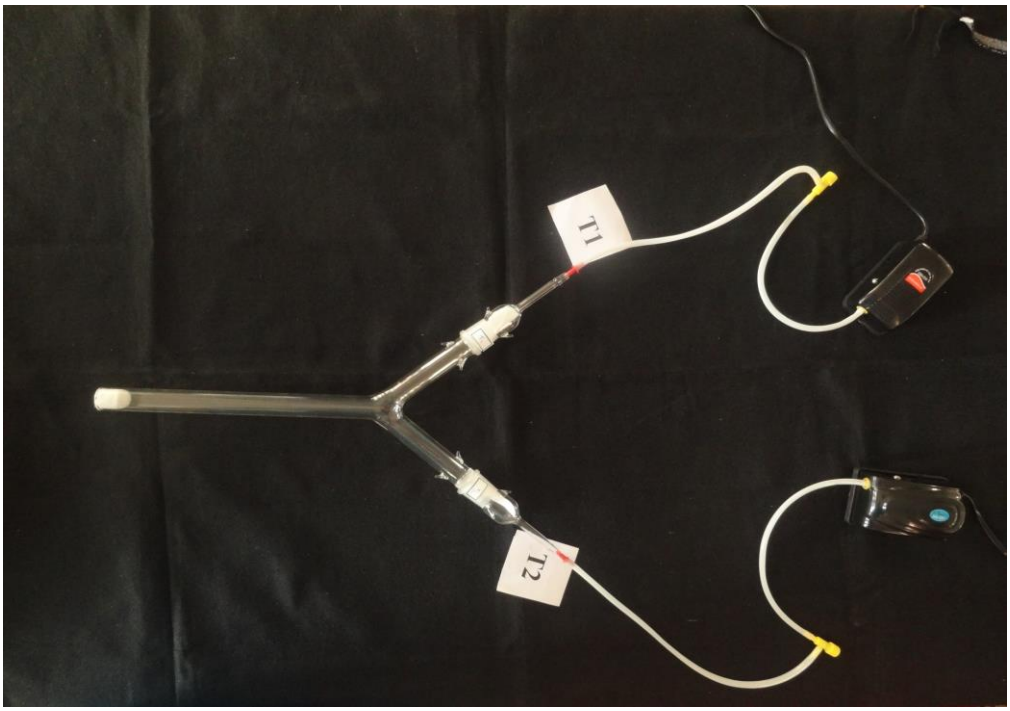


Plate 9. Y–tube olfactometer

humidity of 65-70 % under a 40 W fluorescent lamp. The average airflow through each arm was maintained at 10 L/h.

Synomonal compounds (50 µl) of each brinjal cultivar was taken in a Whatman filter paper of size 2 cm * 1 cm and placed in different arms of the olfactometer. N-hexane (0.5 mL) in a similar strip of filter paper was considered as control and placed in the remaining arm. Ten freshly emerged adult chrysopids were released at the central arena of the olfactometer and observed the response of the chrysopids at every 5 minutes for a period of 30 minutes. The experiment was repeated ten times and data regarding the relative preference of chrysopids towards synomonal extracts were recorded and analyzed.

3.4.4.4.2 *Y- tube Olfactometer Assay*

Based on the previous experiment, the most preferred and the least preferred brinjal cultivar to *C. zastrowi sillemi* were selected for a Y-tube olfactometer assay. The Y-tube olfactometer was made up of glass with 28 cm base length and arm length of 13 cm with 2.5 cm diameter (Plate 9). The olfactometer was kept at a temperature of 27 ^{0}C and relative humidity of 65 to 70 % under a 40 W fluorescent lamp.

The synomonal extracts of mealybug infested and uninfested plants were tested to find out the relative response of *C. zastrowii sillemi* to the volatiles emanated from the plants. Synomonal extracts (50 µl each) were taken in separate filter paper bits and placed inside the two arms of the olfactometer. The individual adult chrysopid was released at one end of the base tube and given 4 minutes to walk towards the end of the olfactometer arm. The choice made by the chrysopid was recorded as it crossed about 4 cm in an olfactometer arm after the division of the base tube and remained for about 20s in the odour source. After testing 5 chrysopids, the olfactometer was washed with ethanol, rinsed using distilled water and dried in a hot air oven and the positions of odour sources were exchanged to avoid any bias in the experiment. The experiment was repeated with another 5

chrysopids and altogether considered as a single replication. The olfactometer assay was replicated 10 times.

3.4.4.5 *Extraction of Kairomones from C. insolita*

The kairomonal compounds emanated from the mealybug body was extracted by immersing mealybug (1g) in 10 mL of HPLC grade hexane. The hexane extract was placed in a shaking water bath at a temperature of 28 ^{0}C for 2 h and later at a temperature of 50 ^{0}C for 20 minutes. The hexane extract was filtered through a Whatman No. 1 filter paper and passed through a silica gel column of 60 to 120 mesh size. The eluted compounds were distilled at 60 to70 ^{0}C in a rotary vacuum flash evaporator and the leftover residue was collected by rinsing the flask with HPLC grade hexane into a small glass vial. The compound was stored at $^-$80 ^{0}C in a low-temperature cabinet (Trang, 2008).

3.4.4.6 *Response of C. zastrowi sillemi to Kairomonal Compounds of C. insolita*

The response of *C. zastrowii sillemi* adults towards the kairomonal compounds of *C. insolita* was evaluated in a Y-tube olfactometer. The olfactometer was kept at a temperature of 27 ^{0}C and relative humidity of 65 to 70 % under a 40 W fluorescent lamp. The kairomonal compound (50µl) was taken in a Whatman filter paper bit of size 2 cm * 1 cm and placed in one arm while n-hexane was taken as the control in another arm of the olfactometer. The experiment was conducted as per the procedure mentioned in 2.3.5.4.2.

3.4.4.7 *Identification of Synomonal and Kairomonal Compounds Using Gas Chromatography-Mass Spectrometry*

The synomonal and kairomonal compounds were identified using Gas Chromatography-Mass Spectrometry (GCMS) at Centre for Analytical Instrumentation Kerala (CAI-K), KFRI, Thrissur.

The synomonal and kairomonal extracts were concentrated using a vacuum concentrator and injected into the column and analyzed using GCMS. The analysis was carried out on a Shimadzu GC-MS QP2010S equipment with

Rxi 5SilMS column (Length-30m, ID- 0.25mm, Film Thickness-0.25micrometre). The carrier gas used was helium with a flow rate of 0.98 mL /minute. The column temperature was programmed to 80°C with an initial temperature of 70°C. The injector temperature was maintained at 260°C and the detector temperature was programmed at 300°C, followed by a linear programmed temperature from 70 to 280°C at a rate of 10°C/minute, operating in electron impact mode.

The mass spectra of unknown compounds were compared with those in the Wiley 8 Spectral Data Base and NIST 11 database.

3.5 STATISTICAL ANALYSIS

The data on morphological parameters of the mealybug *C. insolita* collected from different locations were subjected to ANOVA using WASP 2.0 statistical software. Multivariate analysis (Principal component analysis and Canonical Displacement Analysis) was conducted to identify the genetic difference between the populations using GRAPES software.

The data on field experiment was subjected to ANOVA after proper transformations using WASP 2.0 software. The mean values were separated by DMRT. Correlation and regression analysis was carried out and multiple regression equations were developed. The data on multi armed olfactometer bioassay was done by ANOVA and Y tube olfactometer was done by paired t test.

Results

4. RESULTS

A comprehensive study on "Mealybugs of the vegetable ecosystems and tritrophic interactions of brinjal mealybugs" was conducted at College of Agriculture, Vellayani during the year 2017-2020 with the objective to identify the mealybugs and their natural enemy fauna in solanaceous and cucurbitaceous vegetables, to carry out the molecular characterization of mealy bugs in brinjal and pumpkin and to find out the tritrophic interactions of mealybugs infesting brinjal. The findings from the present study are furnished below in detail.

4.1 COLLECTION AND IDENTIFICATION OF MEALYBUGS IN SOLANACEOUS AND CUCURBITACEOUS VEGETABLES

4.1.1 Documentation of Mealybugs Infesting Solanaceous and Cucurbitaceous Vegetables in Kerala

The mealybugs infesting solanaceous and cucurbitaceous crops were recorded from the study area and is presented in Table 4.

A total of six mealybug species were recorded from solanaceous and cucurbitaceous crops in Kerala (Plate 10 a - f).

4.1.2 Documentation of Mealybugs Infesting Other Vegetable Crops in Kerala

Besides, four mealybug species were also recorded from other vegetable crops in Kerala and is presented in Table 5 (Plate 11 a-d).

4.1.3 Identification of Mealybug Species Infesting Solanaceous and Cucurbitaceous Crops through Morphological Characterization

The important identifying characters of the mealybugs are given below.

4.1.3.1 *Brinjal Mealybug Coccidohystrix insolita* Green

The adult females of brinjal mealybug *C. insolita* are yellowish with a characteristic long ovisac. The body covered with a little amount of wax and long

Table 4. Mealybug species recorded from solanaceous and cucurbitaceous vegetables in Kerala

Sl. No.	Common name	Scientific name	Family	Host plant family
1	Brinjal mealybug	*Coccidohystrix insolita* Green	Pseudococcidae	Solanaceae
2	Striped mealybug	*Ferrisia virgata* (Cockerell)	Pseudococcidae	Solanaceae, Cucurbitaceae
3	Papaya mealybug	*Paracoccus marginatus* Williams and Granara de Willink	Pseudococcidae	Solanaceae
4	Cotton mealybug	*Phenacoccus solenopsis* Tinsley	Pseudococcidae	Solanaceae, Cucurbitaceae
5	Citrus mealybug	*Planococcus citri* (Risso)	Pseudococcidae	Solanaceae, Cucurbitaceae
6	Jack Beardsley mealybug	*Pseudococcus jackbeardsleyi* Gimpel and Milller	Pseudococcidae	Solanaceae, Cucurbitaceae

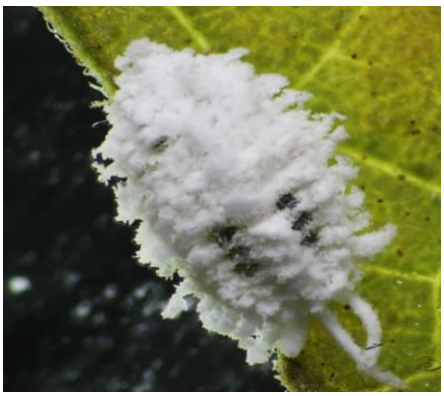
Table 5. Mealybug species recorded from other vegetables in Kerala

Sl. No.	Common name	Scientific name	Family	Host plant family
1	Winged bean Mealybug	*Crisicoccus hirsutus* (Newstead)	Pseudococcidae	Fabaceae
2	Pink hibiscus mealybug	*Maconellicoccus hirsutus* (Green)	Pseudococcidae	Malvaceae
3	Cacao mealybug	*Planococcus lilacinus* (Cockerell)	Pseudococcidae	Convolvulaceae
4	Mango mealybug	*Rastrococcus iceryoides* (Green)	Pseudococcidae	Malvaceae

a.

b.

c.



d.

e.

f.

Plate 10. Mealybugs infesting solanaceous and cucurbitaceous vegetable in Kerala.

a. *Coccidohystrix insolita*, b. *Ferrisia virgata*, c. *Paracoccus marginatus*, d. *Phenacoccus solenopsis*, e. *Pseudococcus jackbeardsleyi* f. *Planococcus citri*.

a.

b.

c.

d.

Plate 11. Mealybugs infesting other vegetables in Kerala.

a.*Crisicoccus hirsutus*, b. *Maconellicoccus hirsutus*, c. *Planococcus lilacinus*, d. *Rastrococcus iceryoides*

filaments. The nymphal stages are yellowish with a pair of dark spots on the thorax and two pairs of spots on the abdomen.

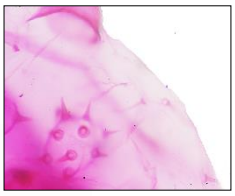
Slide mounted specimen of the adult female is ovoid with 2.24 mm length and 1.42 mm width (Plate 12). Antenna is 420 µm long with nine segments. A total of 17 pairs of cerarii are present, situated on sclerotized regions in the body surface with multiple conical setae. The cerarii on anal lobe contains 5 to 6 conical setae whereas anterior cerarii with 9-10 conical setae. Ocular and preocular cerarii are present, but with smaller size, whereas the cerarii on the metathoracic region is comparatively larger than other cerarii. Cerarii is also present on the dorsal region in between prothorax and abdominal segment VII and submedial cerarii is observed from head to abdominal segment V. Another set of dorsal cerarii is also present in the region of mesothorax and metathorax, which is situated in between medial and submedial cerarii.

Legs are well developed with flagellate tarsal digitules and a claw with a denticle. The length of hind tibia + tarsus is 410.00 µm and hind trochanter + femur is 87.67 µm and the ratio is 1.25. The ratio of hind tibia to tarsus is 2.59. Translucent pores are absent in legs. Circulus is absent. A pair of posterior ostioles are present in the submedial region of the body.

Multilocular disc pores are numerous, distributed on abdominal segment III to VII of ventral surface. Trilocular pores are not abundant. Quinquelocular pores present. Oral collar tubular ducts present. Oral rim tubular ducts absent. Flagellate setae of 35.75 µm length are present in the ventral side.

The dorsal surface contains 33.92 µm long small lanceolate setae. Oral collar tubular ducts are absent. Multilocular disc pores in groups of 2-5 are present on abdominal segment VII. Trilocular pores are uniformly dispersed over the surface. Anal lobes are well developed, anal ring of 79.67 µm wide is present at the apex of the abdomen. The anal ring contains two rows of cells and six setae. The length of setae is about 118.33 µm.

i



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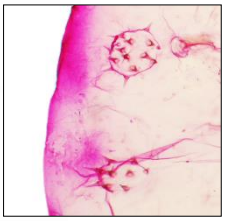

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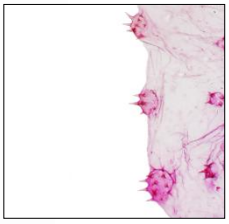

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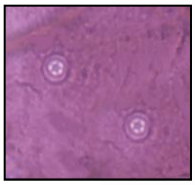

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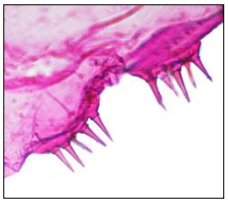
(25X)



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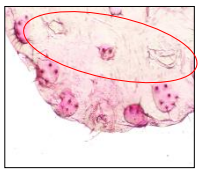


viii

Plate 12. *Coccidohystrix insolita*

i)Antenna with 9 segments (100X), ii) Anterior cerarii with 9-10 conical setae (100X), iii) Tarsal digitules flagellate (100 X), iv)17 pairs of cerarii, situated on sclerotized prominences with multiple conical setae (100X), v)Claw with a denticle (400X), vi) Cerarii on metathoracic region is comparatively larger to other cerarii (100X), vii) Quinquelocular pores present (400 X), viii)A pair of posterior ostioles on the submedial region of the body (100X), ix)Anal lobe cerarii with 5 - 6 conical setae (100X)

4.1.3.2 *Striped Mealybug Ferrisia virgata* (Cockerell)

The adult female mealybugs are elongate to oval, greyish with a white waxy coating over the body. Two long tail- like filaments are present on the posterior part of the body. Lateral filaments are absent. Dark spots or stripes are present in the submedian region of the body. Usually the body covered with long glassy filaments. Eggs and nymphal stages are yellowish in colour.

The slide-mounted specimen of the adult female is elongated to oval, 3.84 mm long and 2.04 mm wide (Plate 13). Antenna is 540 µm long with 8 segments. Cerarii is often associated with two-three conical setae and numerous trilocular pores. Only one pair of cerarii is present in the anal lobes.

Legs are well developed. The length of hind tibia + tarsus is 530 µm and hind trochanter + femur 430 µm and the ratio is 1.23. The ratio of length of hind tibia to tarsus is 2.78. Digitules in the tarsus are capitate. Claws are robust without a denticle. Translucent pores are present in hind coxa, femur and tibia. Both anterior and posterior pair of ostioles are present. Circulus of 120 µm width is present in between abdominal segment III and IV, which is divided by a transverse line.

Dorsal setae are small, 87.5 µm long. Dorsal tubular ducts are long, slender with 2-4 setae around the rim orifice and with discoidal pores. The size of the rim is 11.25 µm which is larger than the size of the multilocular disc pore (8.75 µm). Setae on the ventral surface are slender 117.5 µm long. Trilocular pores are uniformly dispersed over the ventral surface. Multilocular disc pores are arranged in rows starting from abdominal segment VI and VII. Oral collar tubular ducts are present in groups in the ventral margins of abdominal segments. Oral rim ducts are absent. Anal lobes are well developed. Anal ring is 130 µm wide with 6 setae.

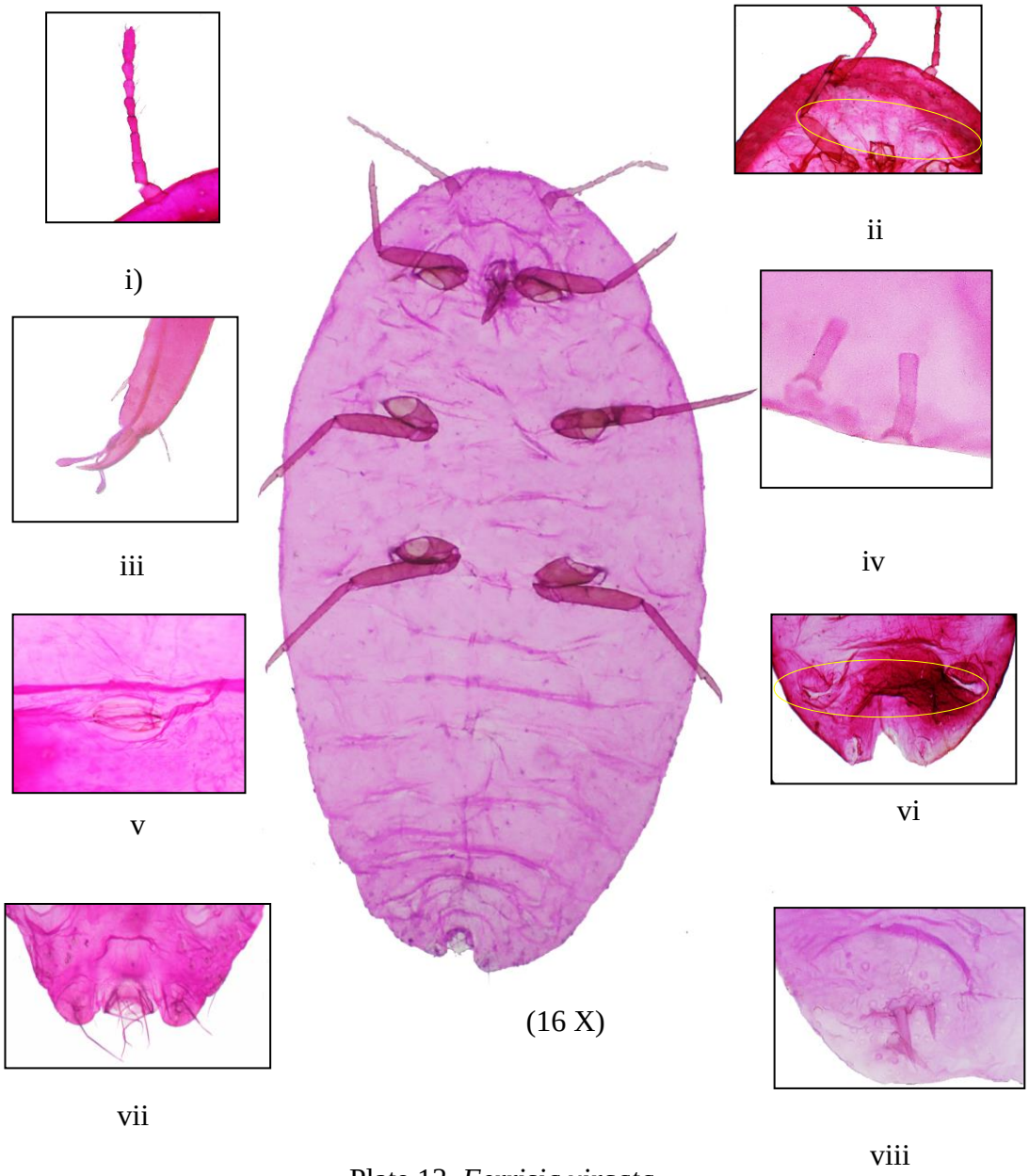


Plate 13 .*Ferrisia virgata*

i)Antenna is with 8 segments (100X), ii)Anterior pair of ostioles are present (100X), iii)Digitules in the tarsus are capitate. Claws are robust without a denticle (400 X), iv)Dorsal tubular ducts are long, slender with 2-4 setae around the rim orifice and with discoidal pores (400X), v) Circulus is present in between abdominal segment III and IV, which is divided by a transverse line (400X), vi)Posterior pair of ostioles are present (100X), vii) Anal lobes are well developed (100 X), viii) Only one pair of cerarii is present in the anal lobes. Cerarii is often associated with two- three conical setae and numerous trilocular pores (100 X)

4.1.3.3 *Papaya Mealybug Paracoccus marginatus Williams and Granara de Willink*

The adult female of *P. marginatus* is elongate - oval and pale yellowish. The body is often covered with a white waxy coating and lateral wax filaments are present. The eggs are yellowish protected inside an ovisac secreted by the adult female.

The slide-mounted specimen of adult female is 2.04 mm long and 1.28 mm wide (Plate 14). Antenna is 550 mm long with 8 segments. It possesses 17 cerarii, which bear mostly two lanceolate conical setae. Cerarii near head and thorax possess 3 - 4 conical setae. Cerariian setae are usually thicker than setae present on the dorsal surface. Axillary setae present only in the cerarii near anal lobe.

Legs are well developed. The length of hind tibia + tarsus is 270 µm and hind trochanter + femur 250 µm and the ratio is 1.08. The ratio of length of hind tibia to tarsus is 2. Claws are robust and curved without a denticle. Translucent pores are present on the hind coxa and absent in hind tibia. Tarsus possess knobbed digitules. Circulus is present, which is partitioned by a transverse line. Anterior and posterior ostioles situated on the submedial region of the body.

The dorsal region lacks multilocular pores and oral tubular ducts while trilocular pores are dispersed over the surface. Oral collar tubular ducts are abundant in the ventral surface. Oral-rim tubular ducts are present on the margins of the body. Multilocular pores are present on the abdominal segments. Anal lobes are well developed with an anal lobe bar. Anal ring is 75 µm wide.

4.1.3.4 *Cotton Mealybug Phenacoccus solenopsis* **Tinsley**

The adult female is oval with a white waxy coating over the body. Lateral filaments and two caudal filaments are present. Dark spots are present on thorax and abdomen which resembles a longitudinal band on the dorsal region. Eggs are yellowish-white and the nymphs are light yellowish in color.

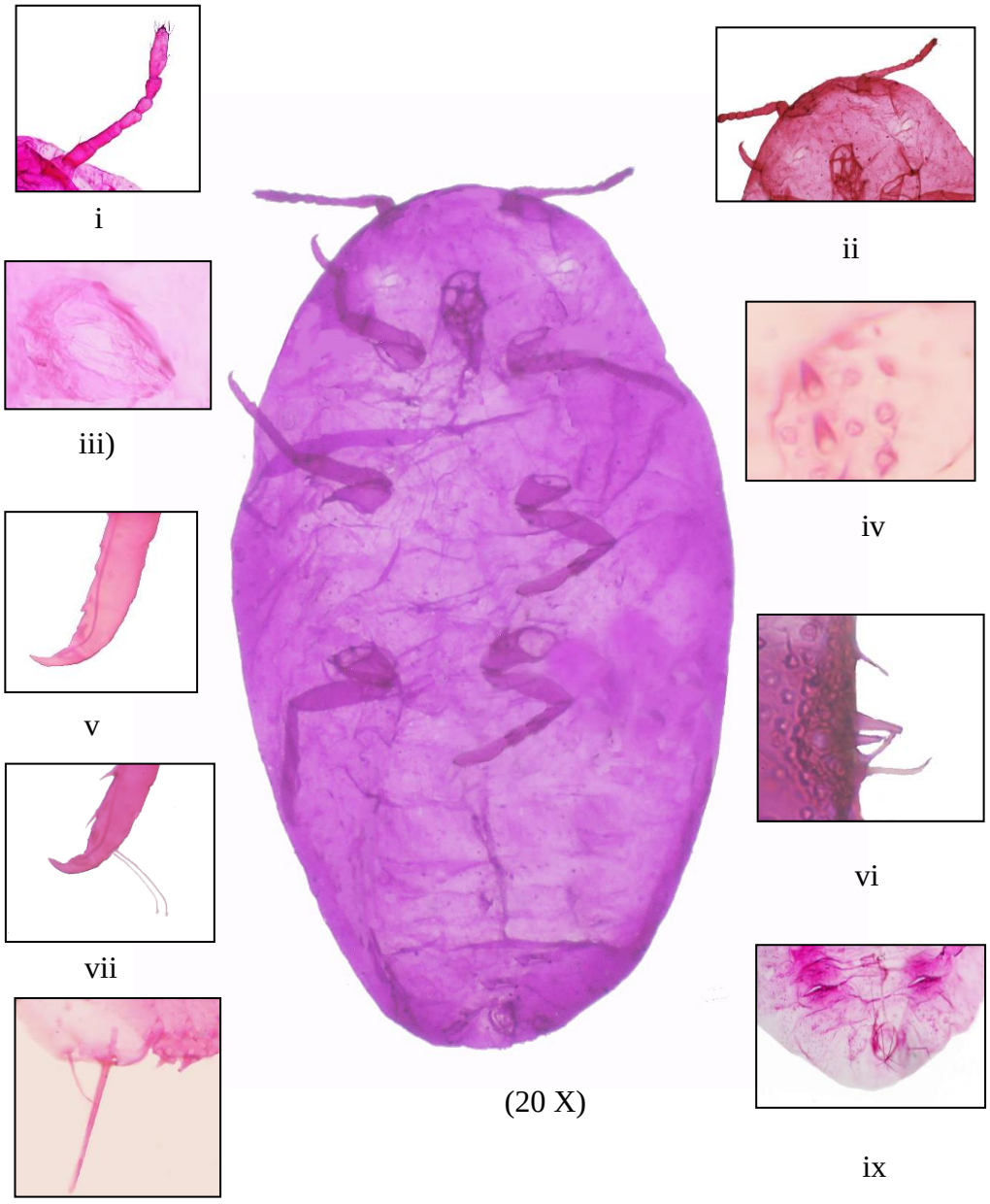


Plate 14. *Paracoccus marginatus*

i)Antenna with 8 segments (100 X) , ii)Anterior ostioles are present (100 X), iii)Circulus is present, which is partitioned by a transverse line (400 X), iv) It possess 17 cerarii, which bear two lanceolate conical setae, Cerariian setae are usually thicker than setae present on the dorsal surface(400X), v)Claws are robust and curved without a denticle (400 X), vi)Axillary setae present only in the cerarii near anal lobe (400 X), vii)Tarsus possess knobbed digitules (400 X), viii)Anal lobes are well developed with an anal lobe bar (400 X), ix) Posterior ostioles are present (100 X)

The body of the slide-mounted specimen of the adult female is elongated to oval 3.34 mm long and 2.04 mm wide (Plate 15). Antenna is 560 µm long with 9 segments. A total of 18 cerarii are present on the body margin. Each cerarii bears two conical setae and trilocular pores around it.

Legs are well developed. The length of hind tibia + tarsus is 480 µm and hind trochanter + femur 430 µm and the ratio is 1.12. The ratio of length of hind tibia to tarsus is 2.69. Flagellate digitules are observed in the tarsus while digitules in the claw are knobbed. Claw often possess a denticle. Translucent pores are present in the hind femur and hind tibia. Circulus is of 80 µm long, 240 µm wide, present in between abdominal segments III and IV, which is large and flaccid. Anterior and posterior pair of ostioles are present.

Small, lanceolate 20 µm long setae are observed on the dorsum. Oral rim ducts, oral collar tubular ducts and multilocular disc pores are absent in the dorsal surface. Trilocular pores are uniformly distributed over both surfaces. Quinquelocular pores are absent.

Ventral setae are 95 µm long. The ventral region is characterized by the presence of multilocular disc pores, dispersed in rows in abdominal segments. Oral collar tubular ducts are abundant whereas oral rim duct is absent in ventral region. Anal ring is well developed with a diameter of 92.5µm with two rows of cells and six setae.

4.1.3.5 *Citrus Mealybug Planococcus citri* (Risso)

The body of the adult female is oval to round and greyish. A thin layer of white wax is covering the body. The median region of dorsum contains a thin longitudinal stripe. The lateral filaments are present on the body region. The lateral filaments on the posterior region are slightly longer than other filaments. Eggs are light yellowish protected inside a white cottony ovisac. The nymphs are light yellowish in color.

Slide mounted specimen of adult female is 3.2 mm long and 1.68 mm wide (Plate 16). Antenna is 340 µm long with 8 segments. A total of 18 cerarii are

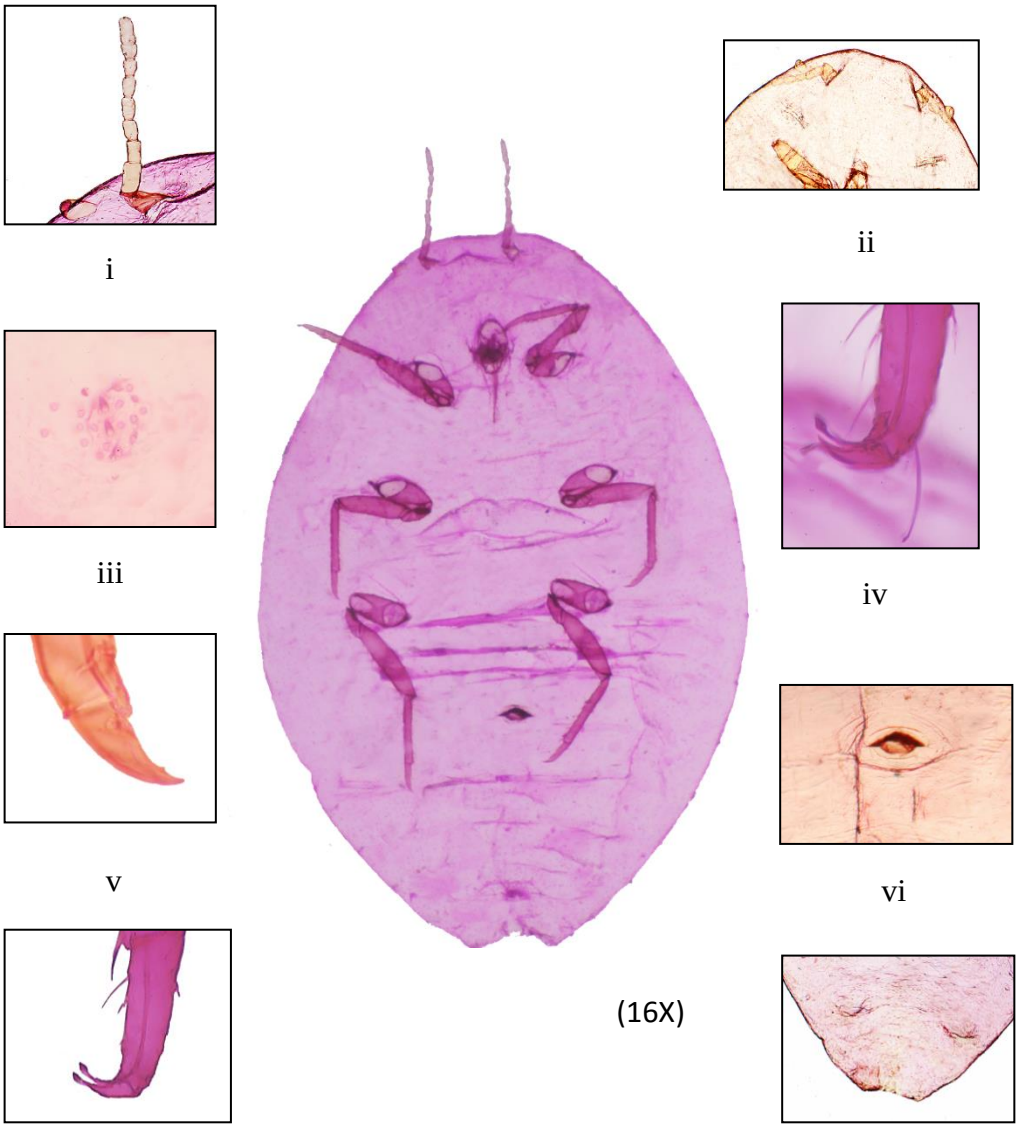


Plate 15. *Phenacoccus solenopsis*

i)Antenna with 9 segments (100X), ii)Presence of anterior pair of ostioles (100 X), iii)A total of 18 cerarii are present on the body margin. Each cerarii bears two conical setae and trilocular pores around it (400 X), iv)Flagellate digitules are observed in the tarsus (400 X), vi)Large and flaccid circulus (100X), vii)Knobbed digitules are observed in the claw (400 X), viii)Presence of posterior pair of ostioles (100 X).

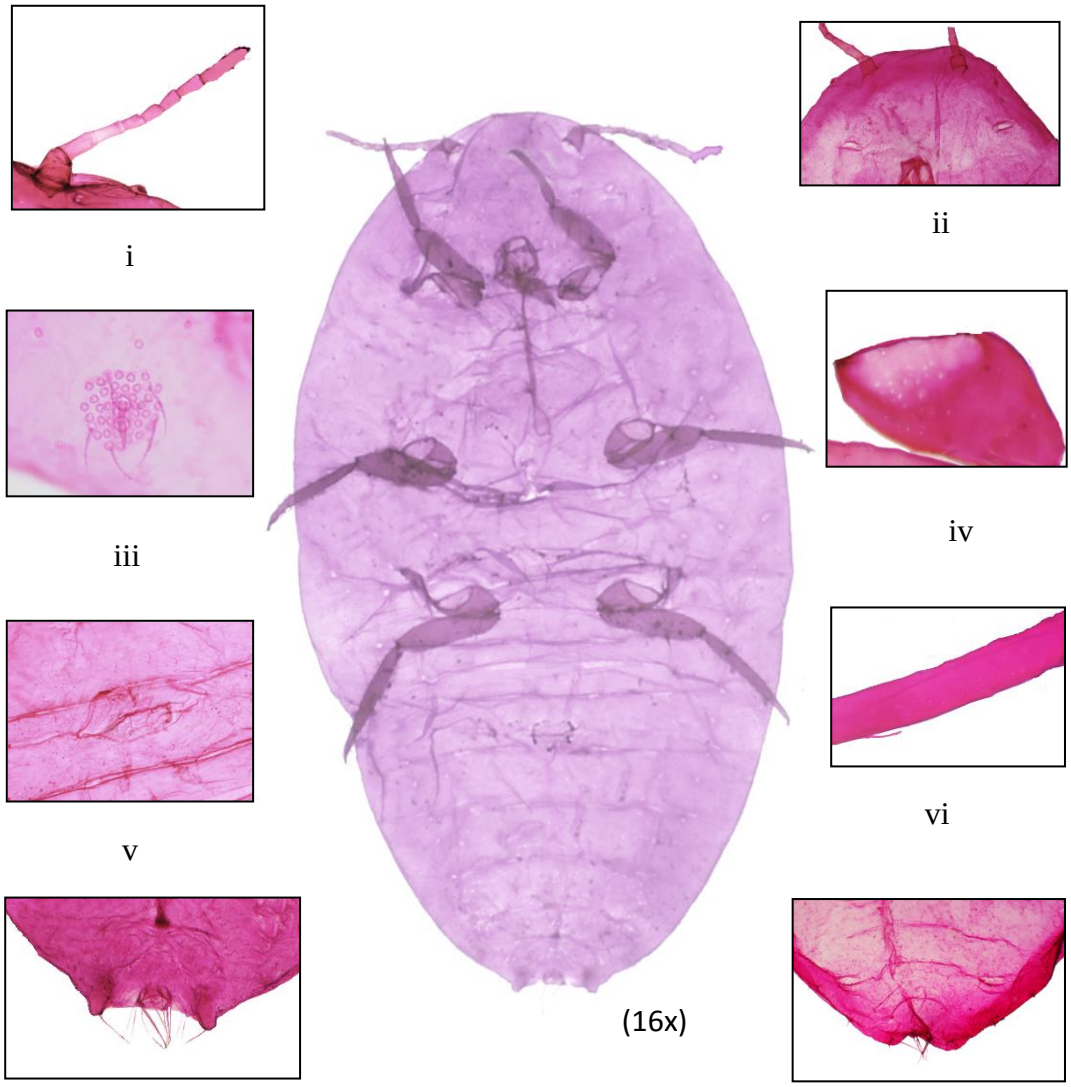


Plate 16. *Planococcus citri*

i)Antenna with 8 segments (100 X), ii)Anterior pair of ostioles are present (100X), iii)A total of 18 cerarii is present on the body surface, each with two conical setae and several trilocular pores (400X), iv)Hind coxa contains translucent pores (400 X), v)Circulus is quadrate, often divided by a transverse line (400X), vi)Hind tibia contains translucent pores (400 X), vii) Cisanal setae is comparatively smaller than anal ring setae (100X) , viii)Posterior pair of ostioles are present (100X)

present on the body surface, each with two conical setae and several trilocular pores.

Legs are well developed. The length of hind tibia + tarsus is 310 µm and hind trochanter + femur 250 µm and the ratio is 1.04- 1.24. Ratio of length of hind tibia to tarsus is 1.9 to 2.00. Hind coxa and tibia contain translucent pores. Both anterior and posterior pair of ostioles are present. Circulus is 320µm wide and 130 µm long, quadrate, often divided by a transverse line.

Dorsal surface bears long flagellate 37.5 µm long setae. The ventral setae are longer than dorsal setae having a length of 45 µm. Oral rim ducts are absent on both surfaces. Oral collar tubular ducts are rarely seen on dorsum, but present on the margins of ventral surface. Multilocular disc pores are absent in the dorsal surface but distributed in rows on ventral surface. Trilocular pores uniformly dispersed on both surfaces. Anal lobes are quite developed into a distinct anal lobe bar. Anal ring is circular with a diameter of 60 µm, bears six setae on it. Cisanal setae are comparatively smaller than anal ring setae.

4.1.3.6 *Jack Beardsley Mealybug Pseudococcus jackbeardsleyi* Gimpel and Millller

The adult female is oval to elongate with light greyish color and covered with a white waxy coating. Lateral wax filaments are present around the body. The caudal filaments are long. White - yellowish eggs are protected inside an ovisac beneath the body. Nymphs are yellowish in color.

Slide mounted specimen of adult female is elongate- oval with 3.38 mm long and 1.86 mm wide (Plate 17). Antenna is 8 segmented with a length of 550 µm. Discoidal pores are present on sclerotized areas around the eyes.

A total of 17 cerarii are present around the body margins. Head cerarii is characterized by 3 conical setae and axillary setae. Anterior cerarii often associated with an oral rim tubular duct. Anal lobe cerarii is situated in a sclerotized area with two conical setae, trilocular pores and auxiliary setae. The penultimate cerarii (C_{17}), also possess auxiliary setae. Preocular cerarii is absent.

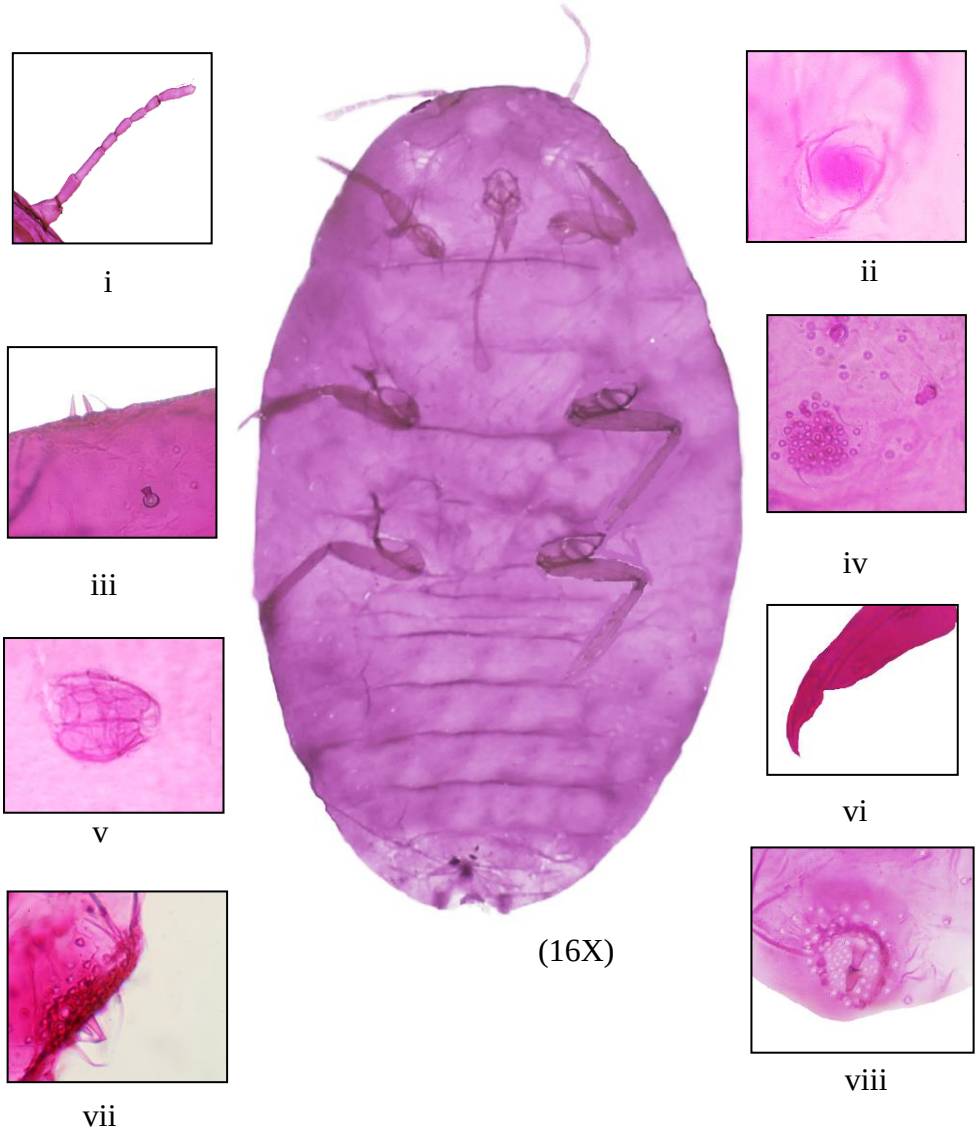


Plate 17. *Pseudococcus jackbeardsleyi*

i) Antenna is 8 segmented (100 X), ii) Discoidal pores are present on sclerotized areas around eyes (400 X), iii)Anterior cerarii often associated with an oral rim tubular duct (400 X), iv)Head cerarii with 3 conical setae and auxillary setae (400 X), v)Circulus is oval and is present in between abdominal segment III and IV (100 X), vi)Claw are stout, curved without a denticle (400 X) , vi)Claw are stout, curved without a denticle (400 X), viii)Anal lobe cerarii is situated in a sclerotized area with two conical setae, trilocular pores and auxillary setae (400X)

Both anterior and posterior pair of ostioles are present. Circulus present in between III and IV abdominal segment is oval, 190 µm long and 210 µm wide.

Legs are well developed. The length of hind tibia + tarsus is 480 µm and hind trochanter + femur is 380 µm and the ratio is 1.26. The ratio of hind tibia to tarsus is 2.69. Translucent pores are present on the hind femur and tibia but absent on hind coxa. Claw are stout, curved without a denticle.

Ventral setae are slender with 50 µm in length. Trilocular pores are uniformly distributed on the dorsal surface. In the ventral surface, multilocular disc pores are numerous and dispersed in the abdominal segments in bands. Oral rim ducts are distributed over the thorax and abdomen. Oral collar tubular ducts of different sizes are present on both surfaces. Quinquelocular pores are absent. Anal bars are absent in anal lobe. The width of anal ring is 80 µm.

4.1.4 Identification of Mealybug Species Infesting Other Vegetable Crops through Morphological Characterization

4.1.4.1 *Winged bean Mealybug Crisicoccus hirsutus* (Newstead)

The adult female is broadly oval, brownish with a white waxy coating over the body. Lateral filaments are present alongside the body. The nymphal stages are light brown in color.

Slide mounted specimen of adult female is broadly oval with a length of 2.15 mm and width of 1.62 mm (Plate 18). Antenna is 410 µm in length and with 8 segments. This species is characterized by the absence of cerarii.

Legs are robust. The length of hind tibia + tarsus is 190 µm and hind trochanter + femur 230 µm and the ratio is 0.83. The ratio of length of hind tibia to tarsus is 1.44. Tarsal digitules are capitate. Claw is robust without a denticle. Hind coxa usually contains translucent pores. Sometimes hind femur and tibia also bear translucent pores. Circulus is 100 µm wide, oval in shape, which is divided by a transverse line in the middle. Anterior and posterior pair of ostioles are present.

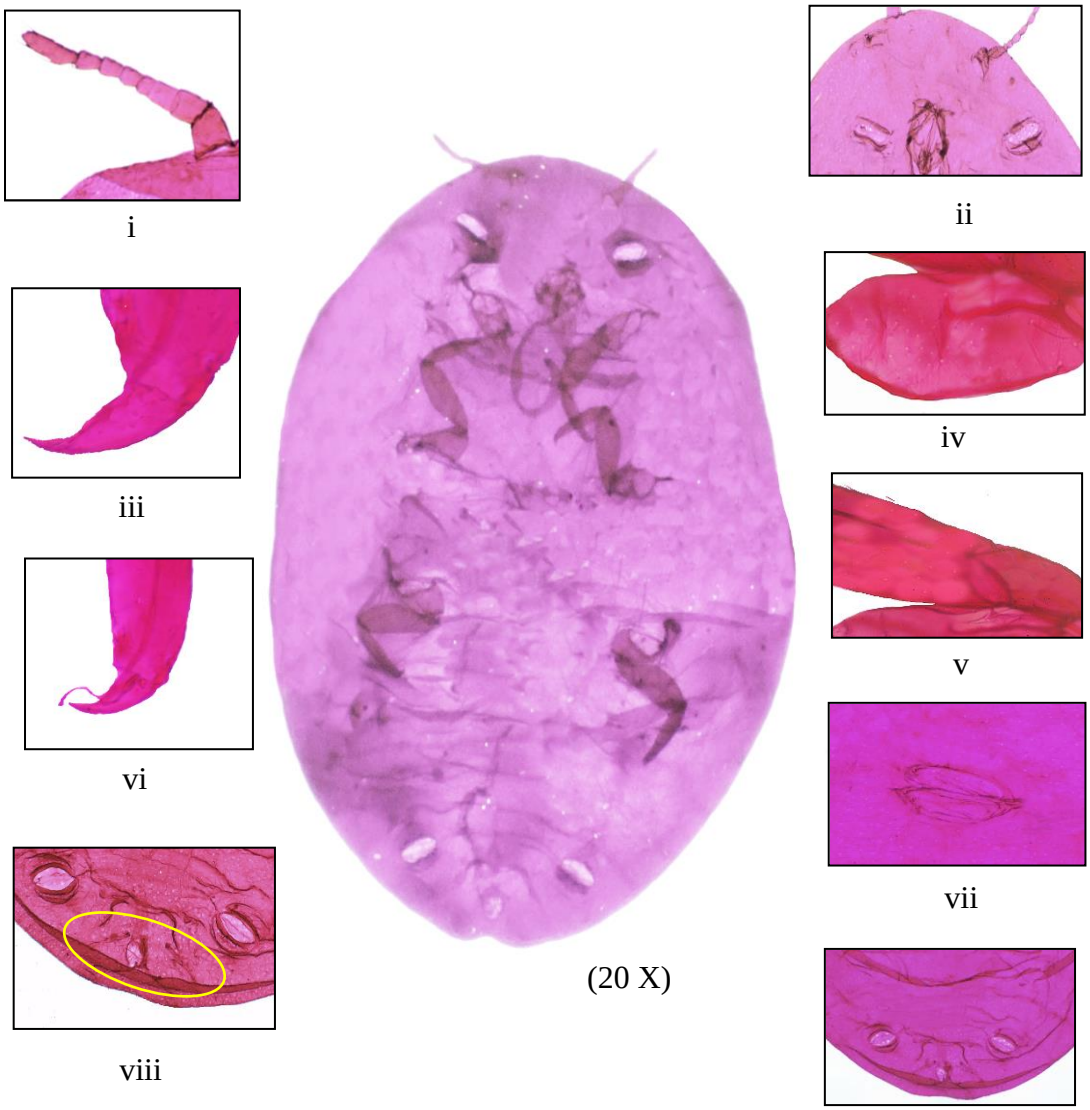


Plate 18. *Crisicoccus hirsutus*

i)Antenna is of 410 µm length and with 8 segments, ii)Anterior pair of ostioles are present (100X), iii)Claw is robust without a denticle (400 X), iv)Hind coxa usually contain translucent pores (400X), v)Hind femur also bears translucent pores (400X), vi) This species is characterized by the absence of cerarii and oral rim tubular ducts, vii) Circulus is oval in shape, which is divided by a transverse line in the middle.(400 X), viii) Anal lobe bar is visible (100 X), ix)Posterior pair of ostioles are present (100X)

Dorsal setae are flagellate, with 55 µm length and similar setae are also present on ventral surface. Multilocular pores are absent on the dorsal surface whereas it was dispersed in the abdominal segments V-VII of ventral surface. Trilocular pores are uniformly dispersed over the dorsal and ventral surfaces. Oral collar tubular ducts are rare on dorsal surface. In the ventral side, oral collar tubular ducts are present which is smaller than the width of a trilocular pore. Oral rim tubular ducts are absent on both surfaces. Anal lobes are distinguishable, with an anal lobe bar. Anal ring is circular, 77.5 µm wide with 6 setae on it.

4.1.4.2 *Pink Hibiscus Mealybug Maconellicoccus hirsutus* (Green)

Adult female of *M. hirsutus* is oval and the body color varies from pink to brownish red and is covered with a little amount of mealy wax. Lateral or caudal filaments are absent. Eggs are orange to pink covered by an ovisac. Nymphs are light orange to pink or light red.

Slide mounted specimen of adult female is oval with a body length of 2.9 mm and width of 1.76 mm (Plate 19). Antenna is 9 segmented with a length of 390 mm. A total of 4-6 cerarii are present on the abdominal segments. Each cerarii bears two conical setae and several trilocular pores.

Legs are well developed. The length of hind tibia + tarsus is 320 µm and hind trochanter + femur 260 µm and the ratio is 1.23. Tarsal digitules are knobbed. Claw is robust without a denticle. Hind femur and tibia possess translucent pores. Circulus is 110 µm wide, which is oval - quadrate in shape. Both anterior and posterior pair of ostioles are present.

Dorsal setae are flagellate having a length of 55 µm. Ventral setae are slender, 80 µm long. Multilocular disc pores are numerous in the abdominal segments. Trilocular pores are uniformly distributed in the body. Oral collar tubular ducts of two sizes are present. Oral rim tubular ducts are abundant on both surfaces. Anal lobes are not well developed but possess apical setae. Anal lobe bar is present, which broadens to the tip of anal lobe. Anal ring is circular, 70 µm wide with six setae.

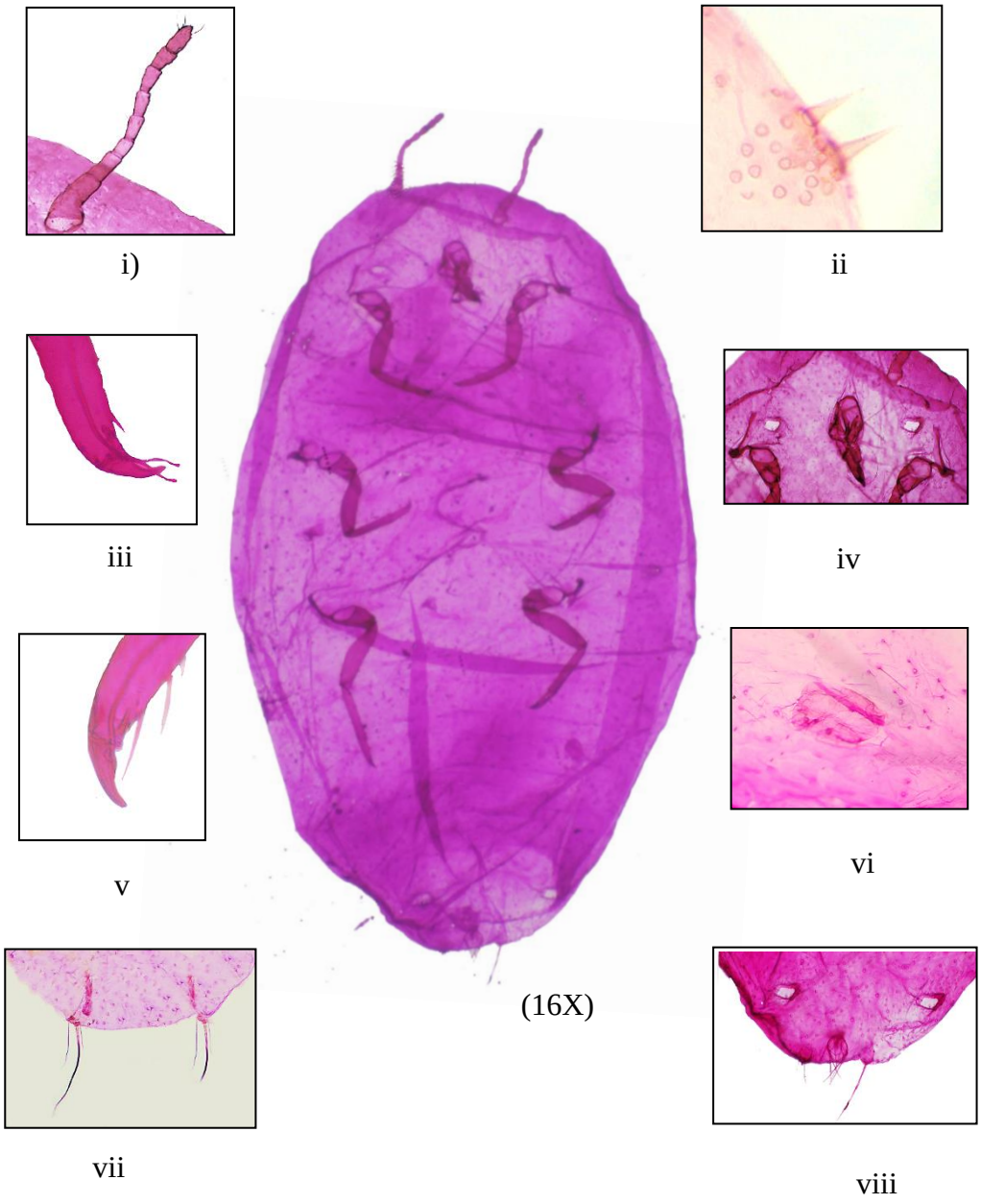


Plate 19. *Maconellicoccus hirsutus*

i)Antenna is 9 segmented (100 X), ii)A total of 4-6 cerarii is present on the abdominal segments, Each cerarii bears two conical setae and several trilocular pores (400X), iii)Tarsal digitules are knobbed (400X), iv)Anterior pair of ostioles are present (100 X), v)Claw is robust without a denticle (400 X), vi)Circulus is oval - quadrate in shape (400 X), vii)Anal lobes are not well developed but possess an apical setae. Anal lobe bar is present which broadens to the tip of anal lobe. (100 X), viii)Posterior pair of ostioles are present (100 X).

4.1.4.3 *Cacao Mealybug Planococcus lilacinus* (Cockerell)

The adult female is round and brownish. The body is covered with a white waxy coating. A conspicuous longitudinal stripe is present on the dorsomedial area. Lateral wax filaments are present. Ovisacs are absent.

Slide mounted specimen of adult female is oval, 2.66 mm long and 2.08 mm wide (Plate 20). Antenna is 410 mm long with 8 segments. A total of 18 cerarii are present, each bear two conical setae and trilocular pores.

Legs are well developed. The length of hind tibia + tarsus is 270 µm and hind trochanter + femur 220 µm and the ratio is 1.22. The ratio of length of hind tibia to tarsus is 1.45. Claws are curved without a denticle. Translucent pores are present on the hind coxa and hind tibia. Circulus is oval and 110 µm wide. It is separated by a transverse line through middle. Anterior and posterior pair of ostioles are present which are characterized by the presence of sclerotized lips and trilocular pores.

Dorsal setae are 52.5 µm long and flagellate. Multilocular pores are rare in dorsal surface. Multilocular pores are abundant on the ventral region, arranged in rows on the abdominal segments. Trilocular pores are evenly dispersed on both sides. Oral collar tubular ducts are present. Oral rim ducts are absent.

Anal lobes are well developed with apical setae, cis anal setae, bar setae and anal lobe bar. Anal ring is circular with a diameter of 70 µm with two rows of cells and six setae. The setae on anal ring is 107.25 µm long which are smaller than the cisanal setae (125µm).

4.1.4.4 *Mango Mealybug Rastrococcus iceryoides* (Green)

The body is round to oval in shape and cream to grey color. A dense coating of mealy wax over the dorsum. Thick lateral filaments are present on margins. Eggs are orange-yellow and protected inside a bulky ovisac. Early instar nymphs are grey and later instars turned to white.

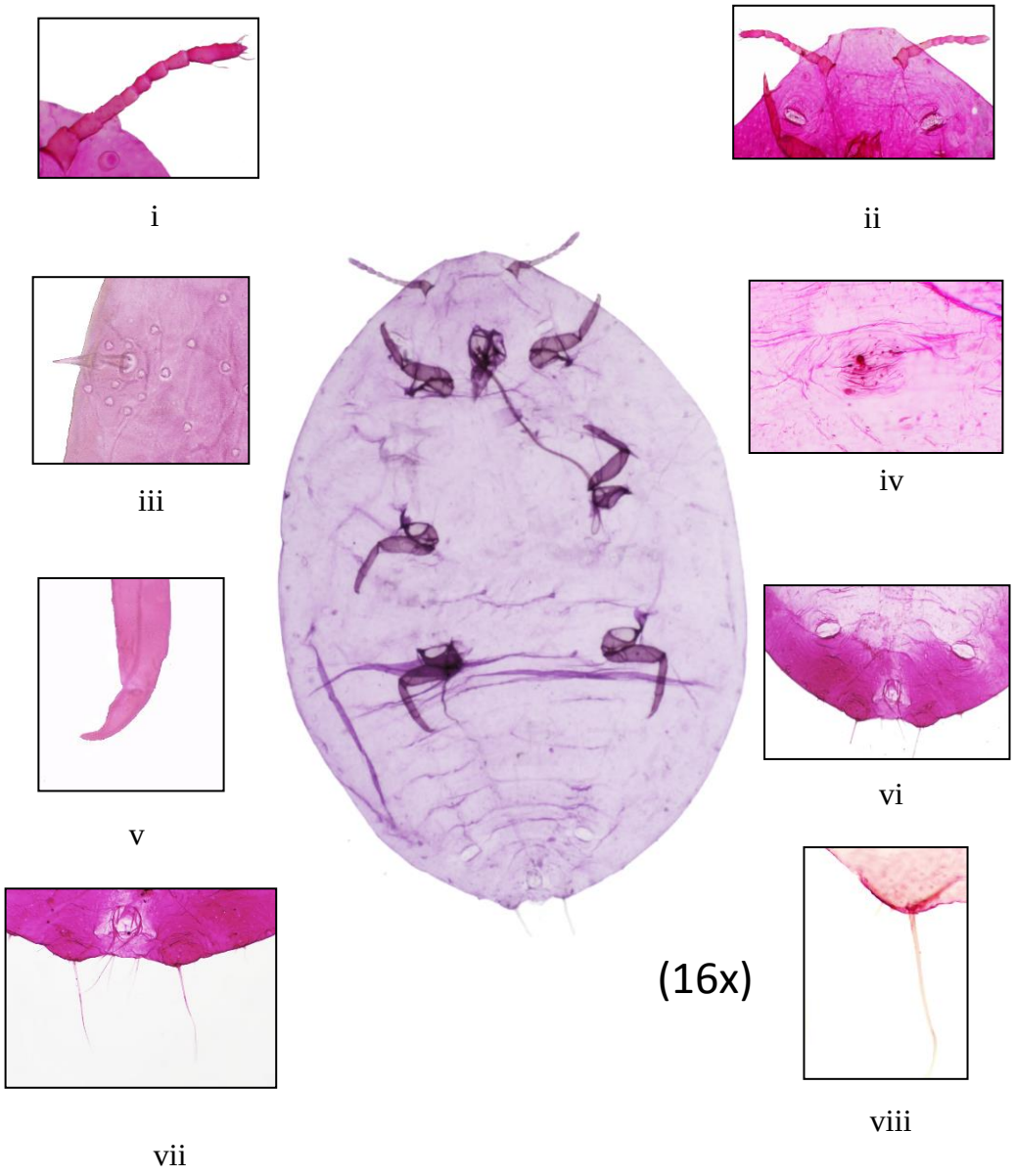


Plate 20. *Planococcus lilacinus*

i)Antenna is with 8 segments (100 X), ii)Anterior pair of ostioles are present (100 X), iii)A total of 18 cerarii is present, each bear two conical setae and trilocular pores (100 X), iv)Circulus is of oval in shape and often separated by a transverse line through the middle. (400 X), v)Claws are curved without a denticle (400 X), vi)Posterior pair of ostioles are present (100 X), vii)Setae on anal ring is smaller than the cisanal setae (125µm) (100 X), viii)Anal lobe bar well developed (400X)

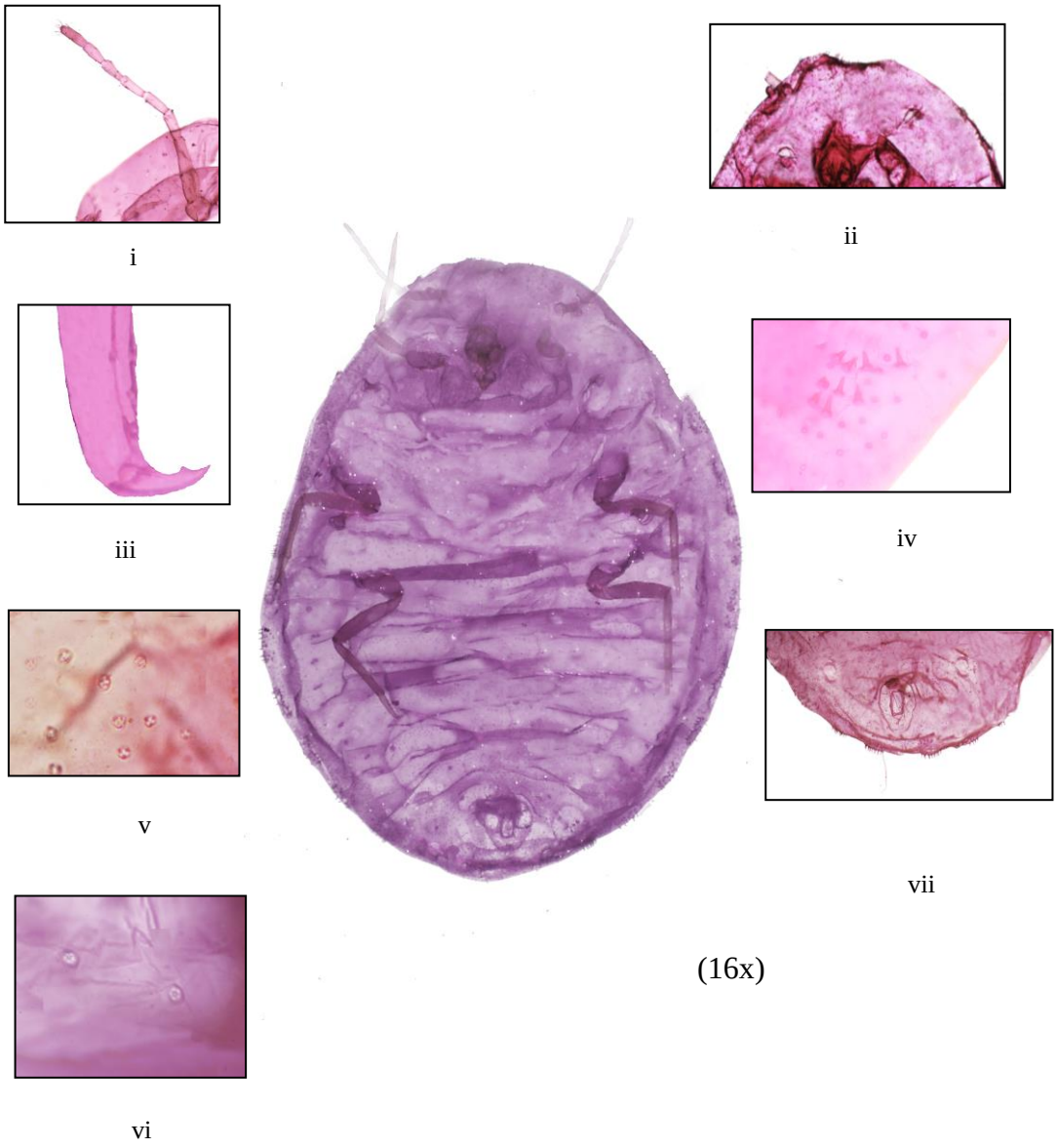


Plate 21. *Rastrococcus iceryoides*

i)Antenna with 9 segments (100 X), ii)Anterior pair of ostioles are present (100X), iii)Claws are robust and curved with a denticle (400X), iv)A total of 17 cerarii are present on body margins. Each cerarius bear more than 10 truncate setae and several large trilocular pores (100X), v) Large sized trilocular pores are present on ventral surface (400X), vi)Quinquelocular pores are present on ventral surface (400X), vii)Posterior pair of ostioles are present (100X).

Slide mounted specimen of adult female is broadly oval 3.44 mm long and 2.7 mm wide (Plate 21). Antenna is 720µm long with 9 segments. A total of 17 cerarii are present on the body margins. Each cerarius bear more than 10 truncate setae and several large trilocular pores.

Legs are well developed. Claws are robust and curved with a denticle. Circulus is 400 µm wide, quadrate in shape, and observed in abdominal segments III. Both anterior and posterior pair of ostioles are present.

Dorsal surface contains small, lanceolate setae with a length of 22.5 µm whereas ventral setae are 30 µm long. Large-sized trilocular pores are present on ventral surface whereas dorsal surface bears similar but small trilocular pores. Multilocular disc pores are present in rows in abdominal segments and also observed in anterior segments. Quinquelocular pores are present on ventral surface. Oral collar tubular ducts of different size are distributed on the body surface. Oral-rim tubular ducts are absent. Anal ring is circular having a diameter of 87.5 µm and six setae.

4.1.5 Distribution of Mealybug Species in Kerala

The distribution of various mealybug species infesting vegetable crops in Kerala is portrayed in Fig. 2a and 2b. The most distributed species in Kerala is striped mealybug, *F. virgata* followed by *P. solenopsis*.

4.1.6 Study of Morphological and Molecular Variations in Brinjal Mealybug *C. insolita*

4.1.6.1 Morphological Variations

The species identity is one of the most important aspects concerning tritrophic interaction studies. So to confirm the species identity, brinjal mealybug collected from different parts of Kerala were studied and the results showed that they exhibited visible morphological variations (Table 6). Out of the 19 characters studied, 13 characters were recorded with significant differences among the various populations.

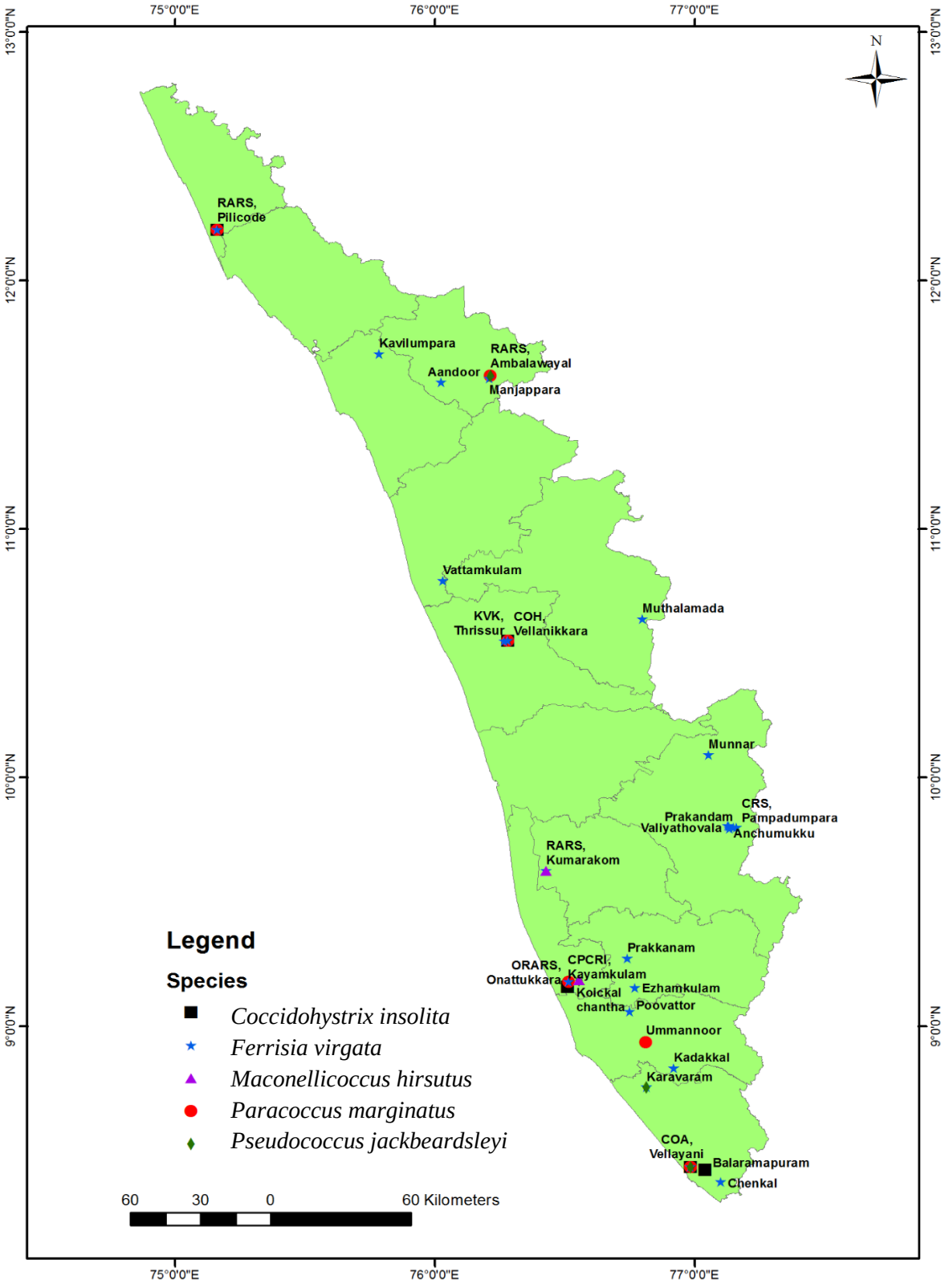


Fig. 2a. Distribution of mealybug species infesting vegetables in Kerala

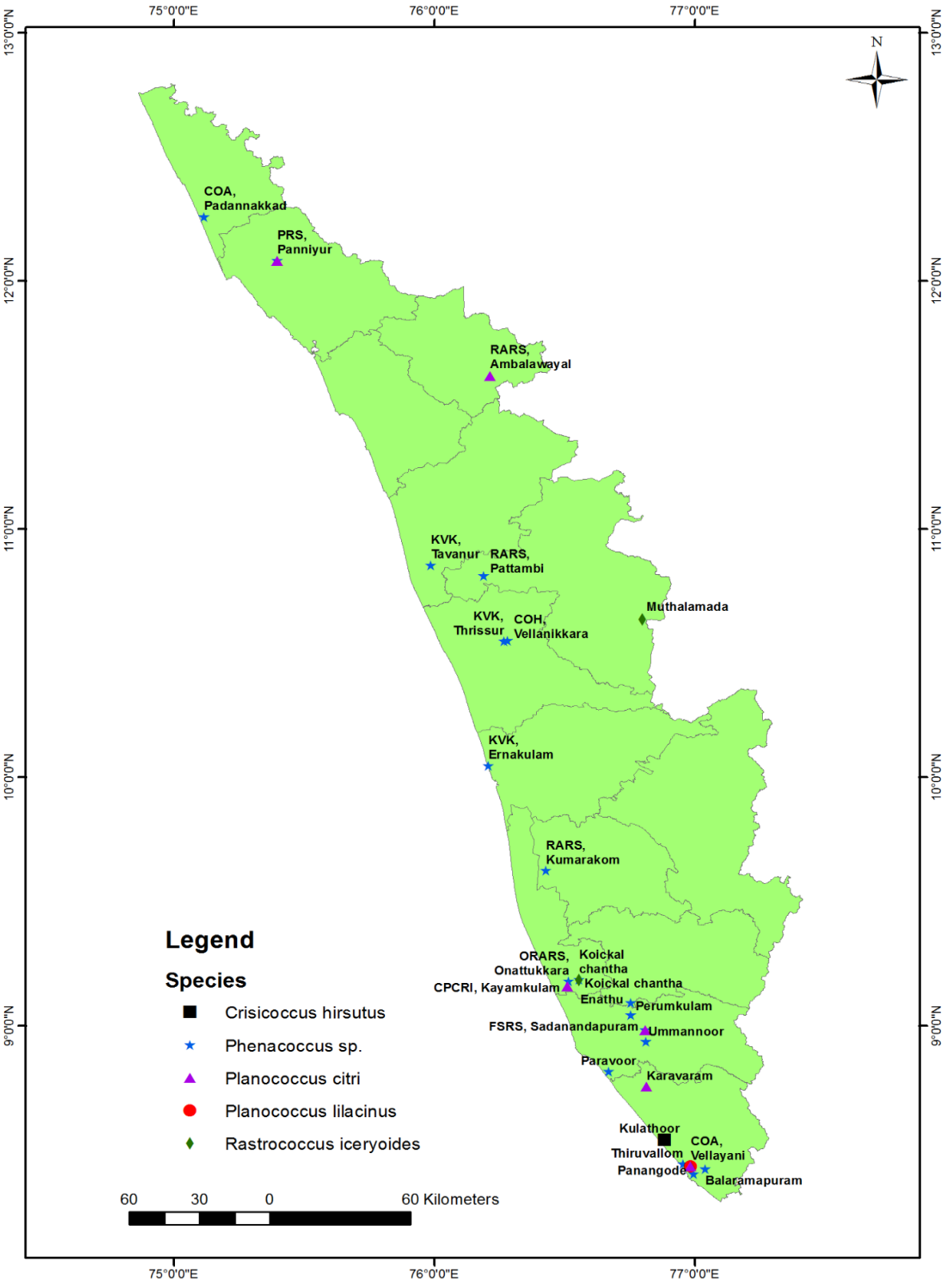


Fig. 2b.Distribution of mealybug species infesting vegetables in Kerala

Mealybug collected from Vellayani showed the highest body length (2520.00 µm) which was statistically superior to all other mealybugs. It was followed by mealybug collected from Thrissur (2390.00 µm) which was statistically different from other mealybugs. The lowest length of body was noted in mealybug collected from Kayamkulam (2060.00 µm) which was statistically on par with the mealybug collected from Balaramapuram (2093.60 µm).

On comparing the width of body, the mealybug collected from Vellayani showed the highest body width (1550.00 µm) which was statistically on par with mealybug from Pilicode (1546.40 µm). The lowest body width was noted in mealybug collected from Kayamkulam (1246.40 µm) which was statistically on par with mealybug from Thrissur (1300.00 µm).

The length of antenna of different populations was compared, of which the mealybug collected from Balaramapuram showed the longest antenna which was statistically on par with Kayamkulam and Thrissur (430.00 µm, 426.80 µm and 425.00 µm respectively). The lowest length of antenna was observed in mealybug collected from Vellayani (405.00 µm) which was statistically on par with Pilicode (413.20 µm).

An appraisal of the similarity between length of hind tarsus revealed that the mealybug collected from Thrissur (125.00 µm) possessed the longest hind tarsus which was statistically superior. The lowest length of hind tibia was observed in mealybug collected from Pilicode (110.00 µm) which was statistically on par with Kayamkulam (110.00 µm), Vellayani (115.00 µm) and Balaramapuram (113.20 µm).

There was significant difference in the length of hind femur of mealybug collected from different parts of Kerala, in which the longest hind femur was observed in mealybug collected from Thrissur (280.00 µm) which was statistically superior to all other mealybugs. The mealybug collected from Pilicode (230.00 µm) showed the lowest length of hind femur which was statistically on par with

Table 6. Morphological variations of brinjal mealybug *Coccidohystrix insolita* collected from different parts of Kerala

Location	Total Length (µm)	Total Width (µm)	Length of the antenna (µm)	Length of hind tibia (µm)	Length of hind tarsus(µm)	Length of hind trochanter (µm)	Length of hind femur (µm)	Length of hind tibia + tarsus (µm)	Length of hind trochanter +femur (µm)	Ratio of length of hind tibia+ tarsus to length of hind trochanter + femur	Ratio of length of hind tibia to tarsus
Pilicode	2160.00	1546.40	413.20	300.00	110.00	93.20	230.00	410.00	323.20	1.28	2.71
Kayamkulam	2060.00	1246.40	426.80	296.80	110.00	90.00	240.00	406.80	330.00	1.24	2.70
Vellayani	2520.00	1550.00	405.00	285.00	115.00	90.00	230.00	400.00	320.00	1.25	2.48
Balaramapuram	2093.60	1466.40	430.00	300.00	113.20	80.00	233.20	413.20	313.20	1.32	2.65
Thrissur	2390.00	1300.00	425.00	295.00	125.00	85.00	280.00	420.00	365.00	1.16	2.39
CD (0.05)	84.922	79.269	13.942	NS	9.173	NS	32.406	NS	NS	0.084	0.199
SE (m)	28.787	26.870	4.726	9.256	3.109	3.629	10.985	11.284	13.705	0.029	0.068
SE (d)	40.710	38.000	6.684	13.090	4.397	5.132	15.535	15.958	19.382	0.040	0.095

Table 6. Morphological variations of brinjal mealybug, *Coccidohystrix insolita* collected from different parts of Kerala (continued)

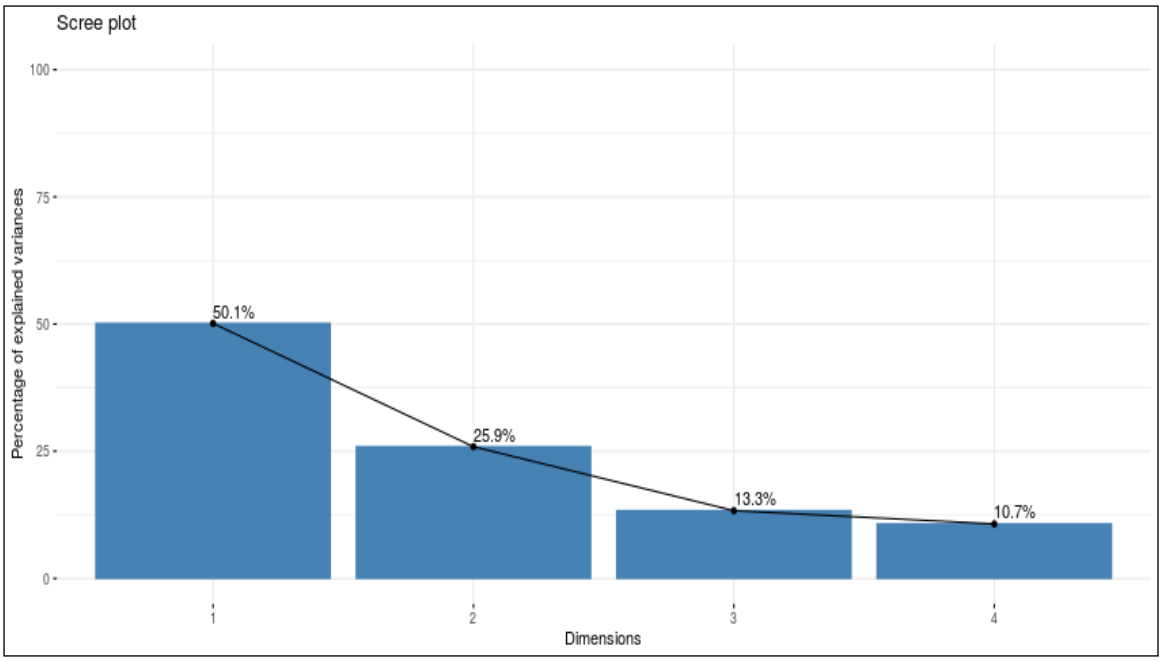
Locations	Total width of anal ring (µm)	Length of anal ring setae (µm)	Length of dorsal setae (µm)	Length of ventral setae (µm)	Length of oral collar tubular duct (µm)	Width of multilocular pore (µm)	Width of quinquelocular pore (µm)	Width of trilocular pore (µm)
Pilicode	68.20	80.80	29.20	40.00	11.25	7.05	5.80	4.85
Kayamkulam	73.20	100.80	32.50	34.20	9.70	7.50	6.70	4.50
Vellayani	90.00	122.50	37.50	28.75	10.00	6.95	5.70	5.00
Balaramapuram	76.80	130.00	36.70	33.30	11.70	7.85	5.45	5.00
Thrissur	90.00	160.00	33.75	42.50	10.00	6.75	6.87	5.00
CD (0.05)	3.618	12.013	4.290	7.220	0.833	NS	NS	NS
SE (m)	1.226	4.072	1.454	2.447	0.282	0.332	0.415	0.152
SE (d)	1.734	5.759	2.057	3.461	0.399	0.470	0.587	0.216

Vellayani (230.00 µm), Balaramapuram (233.20 µm) and Kayamkulam (240.00 µm).

The evaluation of ratio of length of hind tibia + tarsus to length of hind trochanter + femur of mealybug disclosed that the highest ratio was recorded in mealybug from Balaramapuram (1.32) which was statistically on par with Pilicode, Vellayani and Kayamkulam (1.28, 1.25 and 1.24 respectively). The lowest ratio of 1.16 was recorded in mealybug collected from Thrissur. Likewise, the ratio of length of hind tibia to tarsus was also compared and the results showed that the highest ratio was exhibited by mealybug from Pilicode (2.71) which was followed by Kayamkulam (2.70) and Balaramapuram (2.65) which were statistically on par. The lowest ratio was observed in mealybug collected from Thrissur (2.39) which was statistically on par with Vellayani (2.48).

On comparing the total width of anal ring, mealybug collected from Thrissur showed highest width (90.00 µm) which was statistically on par with Vellayani (90.00 µm). It was followed by mealybug collected from Balaramapuram and Kayamkulam with width of 76.80 µm and 73.20 µm respectively whereas the mealybug collected from Pilicode showed lowest width of anal ring (68.20 µm) which was statistically different from all other populations.

The length of anal ring setae was measured and the results showed that the longest anal ring setae were noted in mealybug of Thrissur (160.00 µm) which was statistically superior. This was followed by Balaramapuram and Vellayani (130.00 µm and 122.50 µm respectively). The lowest length of anal ring setae was observed in mealybug collected from Pilicode (80.80 µm) which was statistically different from all other population.



An analysis on the length of dorsal setae disclosed that the longest dorsal setae were observed in mealybug collected from Vellayani (37.50 µm) which was statistically on par with Balaramapuram (36.70 µm) and Thrissur (33.75 µm)

Fig.3 Principal component analysis – scree plot diagram

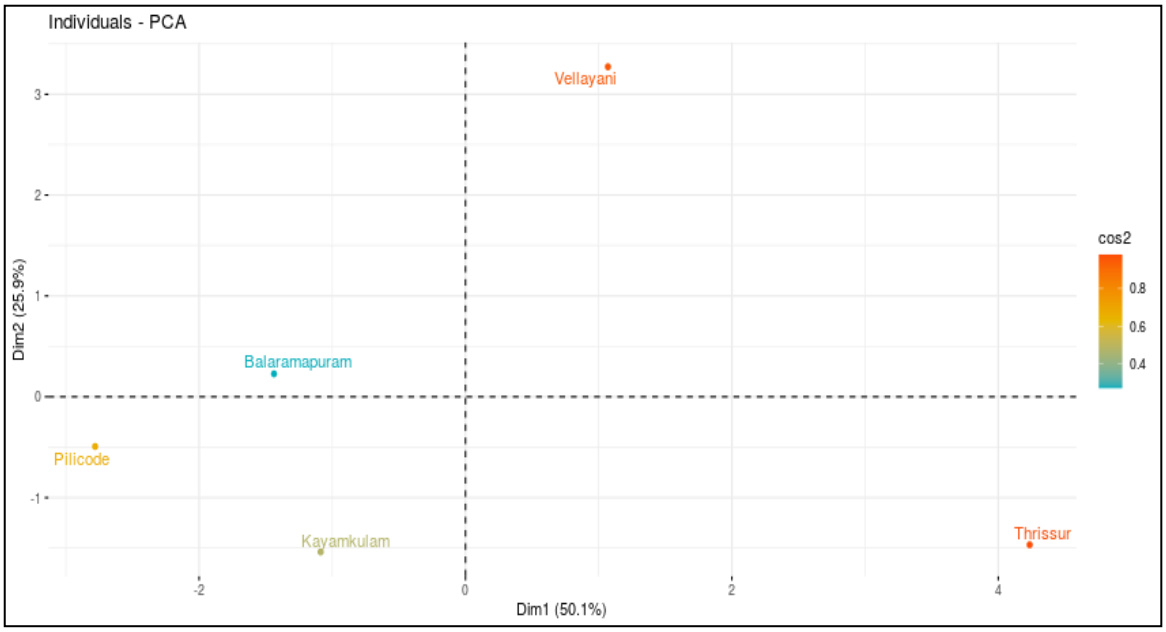


Fig 4. Principal component analysis- biplot diagram

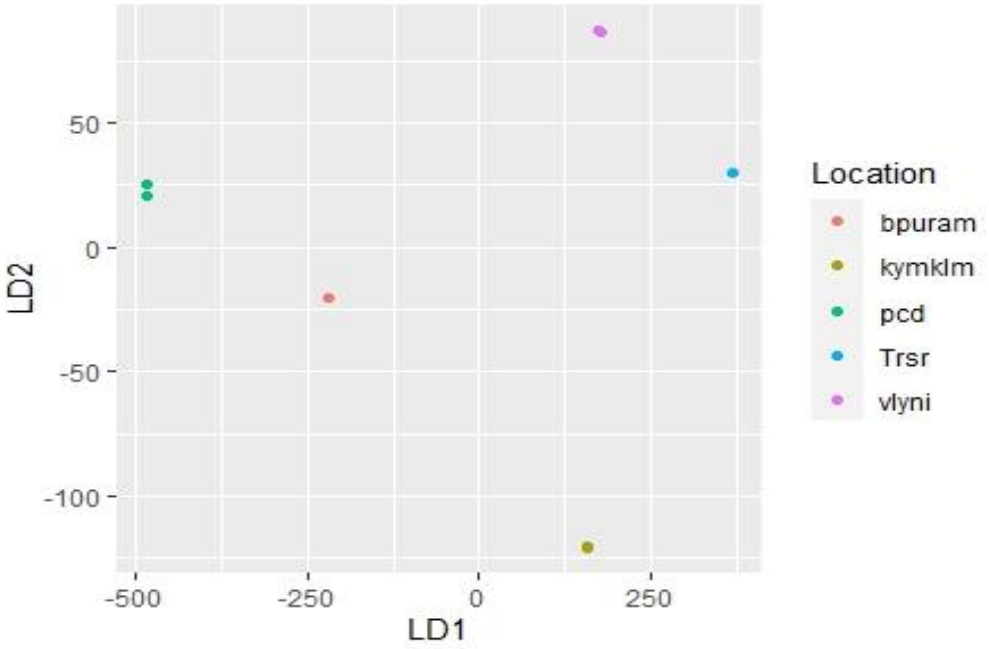


Fig. 5 Canonical discriminant analysis

whereas the lowest length of dorsal setae was noted in mealybug from Pilicode (29.20 µm) followed by Kayamkulam (32.50 µm) which were statistically on par.

Likewise, a comparison on the length of ventral setae of mealybugs revealed that the specimen collected from Thrissur exhibited the longest ventral setal length (42.50 µm) which was statistically on par with Pilicode (40.00 µm). The lowest setal length was observed in mealybug of Vellayani (28.75 µm) which was followed by Balaramapuram and Kayamkulam (33.30 µm and 34.20 µm respectively).

The length of oral collar tubular ducts of mealybug was compared and the results showed that the longest oral collar tubular ducts were recorded in mealybug from Balaramapuram (11.70 µm) which was statistically on par with Pilicode (11.25 µm). The lowest length of oral collar tubular ducts was exhibited by mealybug from Kayamkulam (9.70 µm) which was statistically on par with Vellayani (10.00 µm) and Thrissur (10.00 µm).

The variation in the morphological characters such as length of hind tibia, length of hind trochanter, length of hind tibia + tarsus, length of hind trochanter +femur, width of multilocular pore, width of quinquelocular pore and width of trilocular pore were found to be non-significant among the mealybugs collected from different parts of Kerala.

The scree plot diagram obtained by PCA (Fig. 3) showed that PC_1 exhibited a maximum variation of 50.1 %, PC_2 captured 25.9 %, PC_3 13.3 % and PC_4 10.7 % variation. The first two components accounts for more than 75 % variability and were selected for plotting the biplot. The PCA biplot diagram (Fig. 4) exhibited no overlapping of the components which clearly stated that the populations of mealybug *C. insolita* collected from different parts of Kerala exhibited a great deal of morphological variations. The populations collected from Pilicode and Balaramapuram shared some similarities as the positions were nearby in the biplot diagram. The population of mealybugs collected from Kayamkulam also showed similarity to Pilicode and Balaramapuram population

whereas the population collected from Vellayani expressed variations from the other populations. The population of mealybug *C. insolita* collected from Thrissur exhibited a notable variation from all other population.

The CDA also confirmed the variations exhibited by mealybugs collected from different localities of Kerala (Fig. 5). The plot of the first versus second canonical functions showed 100 % separation of populations.

4.1.6.2 Molecular variations

The molecular characterization of mealybugs collected from different parts of Kerala was carried out to identify the extent of variation exhibited by the *C. insolita* populations.

The genomic DNA of the mealybug was isolated by QIAGEN DNeasy® blood and tissue kit and the PCR products were sequenced at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

The nucleotide sequences obtained are given below.

1) SR1968-MM13-COF- Pilicode

CAGGCTTAAATTTTATTTTACCTGGATTTGGAGTTATATCTCAAATTATAA
ATCAAGAAAATGGAAAAATAGAAATTTTTAGAAAAATTAATATAATTTAT
GCTATAATTTCAATTGGGATTTTAGGTTTTATTGTTTGAGCCCATCACATA
TTTACTATTGGTCTTGATATTGATACTCAACTTTATTTTATATCAGCAACTA
TAATTATTGCTATCCCAACAAGAATTAAAATTTTTAGATGATTAATAACTT
TAAATGGAAAAAAAAACAATAAATCATCAATTAATTTATGATCAATAGGA
TTTATTTTAATATTCACTTTAGGAGGATTAACAGGAATTATTTTATCAAAT
TCAATTATTGACATTAATTTACATGATACCTATTACGTTGTTGCCAGA

2) SR1968-MM13-COF- Kayamkulam

CAGGCTTAAATTTTATTTTACCTGGATTTGGAGTTATATCTCAAATTATAA
ATCAAGAAAATGGAAAAATAGAAATTTTTAGAAAAATTAATATAATTTAT
GCTATAATTTCAATTGGGATTTTAGGTTTTATTGTTTGAGCCCATCACATA
TTTACTATTGGTCTTGATATTGATACTCAACTTTATTTTATATCAGCAACTA

TAATTATTGCTATCCCAACAAGAATTAAAATTTTTAGATGATTAATAACTT
TAAATGGAAAAAAAAACAATAAATCATCAATTAATTTATGATCAATAGGA
TTTATTTTAATATTCACTTTAGGAGGATTAACAGGAATTATTTTATCAAAT
TCAATTATTGACATTAATTTACATGATACCTATTACGTTGTTGCCAGA

3) SR1968-MM8-COF- Vellayani

CAGAGGAAAAATTTTTATTTTACCTGGATTTGGAGTTATATCTCAAATT
ATAAATCAAGAAAATGGAAAAATAGAAATTTTTAGAAAAATTAATAT
AATTTATGCTATAATTTCAATTGGGATTTTAGGTTTTATTGTTTGAGCC
CATCACATATTTACTATTGGTCTTGATATTGATACTCAACTTTATTTTAT
ATCAGCAACTATAATTATTGCTATCCCAACAAGAATTAAAATTTTTAG
ATGATTAATAACTTTAAATGGAAAAAAAAACAATAAATCATCAATTAA
TTTATGATCAATAGGATTTATTTTAATATTCACTTTAGGAGGATTAACA
GGAATTATTTTATCAAATTCAATTATTGACATTAATTTACATGATACCT
ATTACGTTGTTGCAAG

4) SR1968-MM14-COF- Balaramapuram

GGAGGGAAGAGAGGTAGTATGATAGTTATATCGCCCATAAGGCAGGGG
GGTTCTATAATTTTATTTTCCTGGATTTGGAGTTATATCTCAAATTATAAAT
CAAGAAAATGGAAAAATAGAAATTTTTAGAAAAATTAATATAATTTATGC
TATAATTTCAATTGGGATTTTAGGTTTTATTGTTTGAGCCCATCACATATT
TACTATTGGTCTTGATATTGATACTCAACTTTATTTTATATCAGCAACTATA
ATTATTGCTATCCCAACAAGAATTAAAATTTTTAGATGATTAATAACTTTA
AATGGAAAAAAAAACAATAAATCATCAATTAATTTATGATCAATAGGATT
TATTTTAATATTCACTTTAGGAGGATTAACAGGAATTATTTTATCAAATTC
AATTATTGACATTAATTTACATGATACCTATTACGTTGTTGCCAGA.

5) SR1968-MM9-COF- Thrissur

CAAGGATAAATTTTAATTTTACCTGGATTTGGAGTTATATCTCAAATTATA
AATCAAGAAAATGGAAAATATAGAAATTTTTAGAAAAATTAATATAATTT
TTGCTATAATTTCAATTGGAATTTTAGGTTTTATTGTTTGAGCTCATCATAT
ATTTACTATTGGTCTTGATATTGATACTCAACTTTATTTTATATCAGCAACT

ATAATTATTGCTATCCCAACAAGAATTAAAATTTTTAGATGATTAATAACT
TTAAATGGAAAAAAAAACAATAAATCATCAATTAATTTATGATCAATAGG
ATTTATTTTAATATTCACTTTAGGAGGATTAACAGGAATTATTTTATCAAA
TTCAATCATTGACATTAATTTACATGATACCTATTACGTTGTTGCAAGAA
A.

The nucleotide sequences obtained were checked for sequence similarity using nucleotide BLAST at the NCBI database. The results revealed that all the populations belong to the species, *C. insolita*.

4.1.7 Documentation of Host Range of Mealybugs Infesting Solanceous and Cucurbitaceous vegetables

The host range of mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala was documented and the intensity of infestation was recorded from the study area. A total of 113 plants under 73 genera belongs to 31 families were documented as the host plants of mealybugs in Kerala, of which 14 plants were documented as new host reports (Plate 22). The dominant families recorded as host plants of mealybugs were Asteraceae followed by Fabaceae, Malvaceae and Euphorbiaceae in Kerala. Among the plant categories, weeds recorded the highest mealybug infestation followed by vegetables and ornamentals.

The host plants of each mealybug were separately documented and are presented in Table 7 to Table 12.

The brinjal mealybug *C. insolita* recorded from seven host plants belongs to five families in Kerala, of which *Blainvillea rhomboidea* was documented as a new host record (Table 7). Malvaceae and Solanaceae were recorded as dominant plant families infested by *C. insolita* and the plant category, weeds account for the maximum share of host plants. On comparing the level of infestation, most host plants exhibited a medium or high level of infestation by the mealybug.

The striped mealybug, *F. virgata* was recorded from 31 host plants belongs to 16 families, of which seven species were new host records (Table 8).

The new host plants recorded were *Blainvillea rhomboidea*, *Cnidoscolus aconitifolius*, *Microstachys chamaelea*, *Sesbania grandiflora*, *Desmodium triflorum*, *Averrhoa bilimbi* and *Brugmansia suaveolens*. The predominant families of host plants infested by *F. virgata* were Fabaceae, Euphorbiaceae and Solanaceae. Vegetables were recorded with the highest number of hosts followed by weeds and ornamentals. Most of the host plants showed a medium and low level of infestation.

A total of 11 plants under six families were documented as host plants of papaya mealybug, *P. marginatus* in Kerala (Table 9). The family, Malvaceae recorded highest number of host plants followed by Asteraceae. Ornamentals and vegetables were shown maximum susceptibility to *P. margiantus* in Kerala under the study area with most of the plants under high or low level of infestation.

The cotton mealybug, *P. solenopsis*, was recorded as the most dominant mealybug reported from 42 host plants belongs to 17 families (Table 10). Out of this, five species *viz., Spilanthus calva*, *Vernonia cineraria*, *Oldenlandia umbellata*, *Brugmansia suaveolens* and *Talinum fruticosum* were recorded as new host reports. The important plant families infested by *P. solenopsis* were Asteraceae followed by Solanaceae and Malvaceae. Weed plants were more susceptible to *P. solenopsis* infestation followed by vegetables and ornamentals. These host plants showed high level of infestation.

The host range studies of the citrus mealybug *P. citri* revealed 13 plants belongs to 8 families as host plants in Kerala, of which *Pandanus amaryllifolius* were recorded as new host reports (Table 11). The predominant plant family infested by *P. citri* was Fabaceae. Vegetables were more susceptible to mealybug followed by ornamentals which showed a low or incidental level of infestation.

Table 7. Host plants of brinjal mealybug *Coccidohystrix insolita* in Kerala

Sl.No	Botanical name	Family	Plant category	Infestation level
1	*Blainvillea rhomboidea* Cass. *	Asteraceae	Weed	H
2	*Centrosema* sp.	Fabaceae	Weed	M
3	*Hibiscus rosachinensis* L.	Malvaceae	Ornamental	H
4	*Sida rhombifolia* L.	Malvaceae	Weed	M
5	*Cyclea peltata* L.	Menispermaceae	Weed	M
6	*Solanum melongena* L.	Solanaceae	Vegetable	H
7	*Solanum nigrum* L.	Solanaceae	Medicinal	L

Table 8. Host plants of striped mealybug *Ferrisia virgata* in Kerala

Sl.No	Botanical name	Family	Plant category	Infestation level
1	*Amaranthus* sp.	Amaranthaceae	Vegetable	H
2	*Achyranthes aspera* L.	Amaranthaceae	Weeds	L
3	*Mangifera indica* L.	Anacardiaceae	Fruit	M
4	*Nerium* sp.	Apocynaceae	Ornamentals	H
5	*Colcasia esculenta* (L.)	Araceae	Tubers	H
6	*B. rhomboidea**	Asteraceae	Weeds	L
7	*Ipomoea muricata* L.	Convolvulaceae	Vegetable	L
8	*Trichosanthes cucumerina* L.	Cucurbitaceae	Vegetable	L
9	*Cnidoscolus aconitifolius* (Mill.) I.M.Johnst. *	Euphorbiaceae	Vegetable	H
10	*Manihot esculenta* Crantz.	Euphorbiaceae	Tubers	L
11	*Codiaeum variegatum* L.	Euphorbiaceae	Ornamentals	H
12	*Euphorbia pulcherrima* Willd. Ex Klotzsch	Euphorbiaceae	Ornamentals	L
13	*Acalypha indica* L.	Euphorbiaceae	Weed	M
14	*Microstachys chamaelea* (L.)*	Euphorbiaceae	Weeds	H
15	*Cyamopsis tetragonoloba* (L.)	Fabaceae	Vegetable	M

16	*Sesbania grandiflora* (L.)*	Fabaceae	Vegetable	L
17	*Vigna unguiculata* (L.) Walp.	Fabaceae	Vegetable	H
18	*Cajanus cajan* (L.) Millsp.	Fabaceae	Pulses	L
19	*Bauhinia accuminata* L.	Fabaceae	Ornamentals	M
20	*Desmodium triflorum* (L.)*	Fabaceae	Weeds	L
21	*Erythrina* sp.	Fabaceae	Weeds	L
22	*Averrhoa bilimbi* L.*	Oxalidaceae	Fruit	M
23	*Phyllanthus niruri* L.	Phyllanthaceae	Weeds	M
24	*Piper nigrum* L.	Piperaceae	Spices	M
25	*Piper longum* (L.)	Piperaceae	Medicinal	M
26	*Portulaca* sp.	Portulacaceae	Ornamentals	M
27	*Rosa* sp.	Rosaceae	Ornamentals	L
28	*Coffea arabica* L.	Rubiaceae	Beverages	H
29	*Solanum lycopersicum* L.	Solanaceae	Vegetable	M
30	*S. melongena*	Solanaceae	Vegetable	M
31	*Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl *	Solanaceae	Weeds	M

Table 9. Host plants of papaya mealybug *Paracoccus marginatus* in Kerala

Sl.No	Botanical name	Family	Plant category	Infestation level
1	*Cosmos* sp.	Asteraceae	Ornamental	H
2	*Zinnia* sp.	Asteraceae	Ornamental	L
3	*Carica papaya* L.	Caricaceae	Fruit	H
4	*Acalypha indica* L.	Euphorbiaceae	Weed	H
5	*C. cajan*	Fabaceae	Pulses	L
6	*Abelmoschus esculentus*	Malvaceae	Vegetable	M
7	*Hibiscus sabdariffa* L.	Malvaceae	Vegetable	H
8	*Gossypium* sp.	Malvaceae	Fiber	L
9	*Hibiscus mutablilis*	Malvaceae	Ornamental	H
10	*H. rosachinensis*	Malvaceae	Ornamental	H
11	*S. melongena*	Solanaceae	Vegetable	M

Table 10. Host plants of cotton mealybug, *Phenacoccus solenopsis* in Kerala

Sl.No	Botanical name	Family	Plant category	Infestation level
1.	*Amaranthus* sp.	Amaranthaceae	Vegetable	H
2.	*Spinacia oleracea* Linn.	Amaranthaceae	Vegetable	L
3.	*Aerva lanata* (L.)	Amaranthaceae	Weeds	H
4.	*Alternanthera* sp.	Amaranthaceae	Weeds	L
5.	*Eryngium foetidum* L.	Apiaceae	Spice	M
6.	*Cosmos* sp.	Asteraceae	Ornamental	H
7.	*Tagetes* sp.	Asteraceae	Ornamental	H
8.	*Tithonia diversifolia* Hemsl.	Asteraceae	Ornamental	M
9.	*Zinnia* sp.	Asteraceae	Ornamental	L
10.	*Ageratum conyzoides* L.	Asteraceae	Weeds	H
11.	*Ageratum houstonianum* Mill.	Asteraceae	Weeds	H

12.	*Chromalena odorata* (L.)	Asteraceae	Weeds	M
13.	*Eclipta alba* (L.)	Asteraceae	Weeds	M
14.	*Spilanthus calva* DC.*	Asteraceae	Weeds	H
15.	*Synedrella nodiflora* (L.)	Asteraceae	Weeds	L
16.	*Tridax procumbens* L.	Asteraceae	Weeds	L
17.	*Vernonia cineraria* (L.)*	Asteraceae	Weeds	H
18.	*Heliotropium indicum* L.	Boraginaceae	Weeds	H
19.	*Cleome rutidosperma* DC.	*Cleomaceae*	Weeds	M
20.	*Cucurbita pepo* L.	Cucurbitaceae	Vegetable	L
21.	*Euphorbia heterophylla* L.	Euphorbiaceae	Weeds	H
22.	*Euphorbia hirta* L.	Euphorbiaceae	Weeds	L
23.	*Canavalia ensiformis* (L.) DC	Fabaceae	Vegetable	L
24.	*Indigofera* sp.	Fabaceae	Weeds	L
25.	*Gossypium* sp.	Malvaceae	Fiber	L
26.	*H. rosachinensis*	Malvaceae	Ornamental	H
27.	*A. esculentus*	Malvaceae	Vegetable	H
28.	*Sida acuta* Burm.f.	Malvaceae	Weeds	H
29.	*S. rhombifolia*	Malvaceae	Weeds	M
30.	*Boerhavia erecta* L.	Nyctaginaceae	Weeds	H
31.	*Sesamum indicum* L.	Pedaliaceae	Oilseeds	H
32.	*P. niruri*	Phyllanthaceae	Weeds	L
33.	*Scoparia dulcis* L.	Plantaginaceae	Weeds	M
34.	*Portulaca* sp.	Portulacaceae	Ornamental	H
35.	*Ixora coccinea* L.	Rubiaceae	Ornamental	L
36.	*Oldenlandia umbellate* L.*	Rubiaceae	Weeds	M
37.	*Capsicum annuum* L.	Solanaceae	Vegetable	H
38.	*S. lycopersicum*	Solanaceae	Vegetable	H

39.	*S. melongena*	Solanaceae	Vegetable	H
40.	*B. suaveolens**	Solanaceae	Weeds	L
41.	*S. torvum*	Solanaceae	Weeds	H
42.	*Talinum fruticosum* (L.)*	Talinaceae	Vegetable	M

Table 11. Host plants of citrus mealybug, *Planococcus citri* in Kerala

Sl.No	Botanical name	Family	Plant category	Infestation level
1	*Gerbera* sp.	Asteraceae	Ornamentals	L
2	*Mikania micrantha* Kunth.	Asteraceae.	Weeds	I
3	*Momordica charantia* L.	Cucurbitaceae	Vegetable	L
4	*Codiaeum variegatum* L.	Euphorbiaceae	Ornamentals	L
5	*C. ensiformis*	Fabaceae	Vegetable	I
6	*S. grandiflora*	Fabaceae	Vegetable	L
7	*V. unguiculata*	Fabaceae	Vegetable	L
8	*C. cajan*	Fabaceae	Pulses	L
9	*Pandanus amaryllifolius* Roxb.*	Pandanaceae	Spices	I
10	*I. coccinea*	Rubiaceae	Ornamental	L
11	*S. lycopersicum*	Solanaceae	Vegetable	L
12	*S. melongena*	Solanaceae	Vegetable	M
13	*Clerodendrum serratum* (L.)	Verbenaceae	Medicinal plant	L

*New host records

Table 12. Host plants of Jack Beardsley mealy bug *Pseudococcus jackbeardsleyi* in Kerala

Sl. No.	Botanical name	Family	Plant category	Infestation level
1	*S. oleracea*	Amaranthaceae	Vegetable	L
2	*Cordyline terminalis* (L.)	Asparagaceae	Ornamentals	M
3	*I. muricata*	Convolvulaceae	Vegetable	L
4	*Lagenaria siceraria* (Molina) Standl*	Cucurbitaceae	Vegetable	M
5	*C. variegatum*	Euphorbiaceae	Ornamentals	L
6	*A. indica*	Euphorbiaceae	Weed	M
7	*H. rosachinensis*	Malvaceae	Ornamental	L
8	*S. lycopersicum*	Solanaceae	Vegetable	L
9	*S. melongena*	Solanaceae	Vegetable	M

*New host records

a

b

c

d

e

f

g

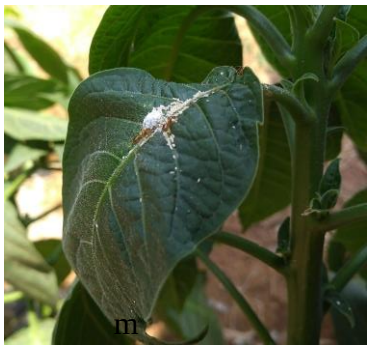
h

Plate 22. New host plants recorded for mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala.

a. *Blanvillea rhomboidea- C. insolita,* b. *Cnidoscolus aconitifolius- F. virgata,* c. *Blanvillea rhomboidea- F. virgata,* d. *Desmodium triflorum -F. virgata,* e. *Sesbania grandiflora -F. virgata,* f. *Brugmansia suaveolens -F. virgata,* g. *Averrhoa bilimbi-F. virgata,* h. .*Microstachys chamaelea-F. virgata*

i

j



k

l

m

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Plate 22. New host plants recorded for mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala.

i.*Spilanthus calva- P.solenopsis,* j. *Vernonia cineraria-P. solenopsis,* k. *Brugmansia suaveolens- P. solenopsis,* l. *Oldenlandia umbellata- P. solenopsis,* , m. *Talinum fruticosum- P. solenopsis,* n. *Pandanus amaryllifolius.- P. citri,*

host record (Table 12). Plant families *viz.,* Euphorbiaceae and Solanaceae contained highest number of host plants. Among the plant categories, vegetables and ornamentals showed the highest number of host plants and most of the host plants showed a low level of infestation.

4.1.8 Documentation of Natural Enemies of Mealybugs infesting Solanaceous and Cucurbitaceous Vegetables

The natural enemies associated with mealybugs were documented from the study area and the details associated with the predators of mealybugs are depicted in Table 13.

4.1.8.1 *Predators*

Twenty species of predators belongs to five families under four orders *viz.* Coleoptera, Lepidoptera, Diptera and Neuroptera were recorded from mealybugs species infesting solanaceous and cucurbitaceous vegetables. The predominant predatory family was coccinellidae with 16 species under six genera and the majority belongs to the genus *Scymnus*. The coccinellids beetles were identified by Dr. J. Poorani, Scientist, NRC-Banana, Trichy.

The coccinellid predator, *Brumoides suturalis* (Fabricius) (Plate 23 a) was observed from two mealybug species *viz., C. insolita* and *P. solenopsis* whereas the predator *Cheilomenes sexmaculata* (Fabricius) (Plate 23b) was recorded from *C. insolita.* The mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant, (Plate 23c) rare in plain areas of Kerala was observed from Balaramapuram of Thiruvananthapuram district which was found to be predated on *C. insolita. C. montrouzieri* was recorded as the dominant predator of *F. virgata* in high altitude regions of Kerala. The coccinellid predators, *Hyperaspis maindroni* Sicard (Plate 23d) and *Pharoscymnus horni* (Wiese) (Plate 23e) were observed as predator of *C. insolita.*

Table 13. Predators recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

Sl. No	Name of species	Order and family	Prey/ associated habit	Location
1	*Brumoides suturalis* (Fabricius)	Coleoptera Coccinellidae	*C. insolita*	Vellayani Balaramapuram
			P. solenopsis	Vellanikkara
2	*Cheilomenes sexmaculata* (Fabricius)	Coleoptera Coccinellidae	*C. insolita*	Balaramapuram
3	*Cryptolaemus montrouzieri* Mulsant	Coleoptera Coccinellidae	*C. insolita*	Balaramapuram
			F. virgata	Valiyathovala
4	*Hyperaspis maindroni* Sicard	Coleoptera Coccinellidae	*C. insolita*	Vellayani Balaramapurm Vellanikkara
5	*Pharoscymnus horni* (Wiese)	Coleoptera Coccinellidae	*C. insolita*	Vellayani Vellanikkara
6	*Scymnus coccivora* Ayyar	Coleoptera Coccinellidae	*C. insolita*	Vellanikkara Balaramapuram
7	*Scymnus* sp.1	Coleoptera Coccinellidae	*C. insolita*	Vellayani
8	*Scymnus* sp.2	Coleoptera Coccinellidae	*C. insolita*	Vellayani
9	*Scymnus* sp. 3	Coleoptera Coccinellidae	*C. insolita*	Vellanikkara
10	*Scymnus* sp. 4	Coleoptera Coccinellidae	*C. insolita*	Vellayani
11	*Scymnus* sp. 5	Coleoptera Coccinellidae	*P. jackbeardsleyi*	Ambalavayal
12	*Scymnus* sp. 6	Coleoptera Coccinellidae	*F. virgata*	Vellayani
13	*Nephus* sp.	Coleoptera Coccinellidae	*Planococcus* sp.	Vellayani
14	unidentified sp.1	Coleoptera Coccinellidae	*F. virgata*	Vellayani
15	unidentified sp. 2	Coleoptera Coccinellidae	*C. insolita*	Vellayani

16	unidentified sp. 3	Coleoptera Coccinellidae	*C. insolita*	Vellayani
17	*Spalgis epius* (Westwood)	Lepidoptera Lycaenidae	*C. insolita*	Vellayani
			Planococcus sp.	
			F. virgata	
			P. solenopsis	
			P. marginatus	
18	*Diadiplosis* sp.	Diptera Cecidomyiidae	*P. jackbeardsleyi*	Vellayani
19	*Cacoxenus* sp.*	Diptera Drosophilidae	*P. jackbeardsleyi*	Ambalavayal
20	*Chrysoperla* sp.	Neuroptera Chrysopidae	*F. virgata*	Vellayani
			C. insolita	

*New host record

The genus *Scymnus* comprised of seven species (Plate 23f - l), of which *Scymnus coccivora* Ayyar was prevalent in the vegetable ecosystem predating on *C. insolita*. Most of the *Scymnus* species were found predating on *C. insolita* whereas certain species were observed in *P. jackbeardsleyi* and *F. virgata*.

A coccinellid predator belongs to the genus *Nephus* (Plate 23m) was recorded from *Planococcus* sp. infesting cocoa plants. Besides, two unidentified species from *F. virgata* infesting chayamansa plants (Plate 23n-p).

The ape fly, *Spalgis epius* (Westwood) (Plate 23q) belongs to the family Lycaenidae (Order: Lepidoptera), a potential predator of mealybug, was recorded from *C. insolita, F. virgata, P. solenopsis, P. marginatus* and *Planococcus* sp. The larval stages of the lycaenid was found as voracious feeder of all stages of mealybug.

A predatory gall midge (Cecidomyiidae: Diptera), *Diadiplosis* sp. (Plate 23 r) was documented as a predator of the mealybug, *P. jackbeardsleyi*. The larval stages of the gall midge act as active predator of mealybugs preferably feeding on egg and early instar nymphs. The specimens were identified by Dr. Netta Dorchin, School of Zoology, Curator of Entomology, The Steinhardt Museum of Natural History, Tel Aviv University, Israel. Another dipteran predator, *Cacoxenus* sp. (Plate 22s) belongs to the family Drosophilidae was also recorded from the mealybug, *P. jackbeardsleyi* and was reported for the first time. The specimen was identified by Dr. Rajendra S. Fartyal, Assistant Professor, Department of Zoology & Biotechnology, Chauras Campus, HNB Garhwal University, Garhwal, Uttarakhand.

A chrysopid predator, *Chrysoperla* sp. (Plate 23t) was observed as a predator of the mealybug, *F. virgata* and *C. insolita* infesting on chayamansa in Kerala. The specimens were identified by Dr. Catherine A Tauber, Department of Entomology, Cornell University, Ithaca, New York, USA.

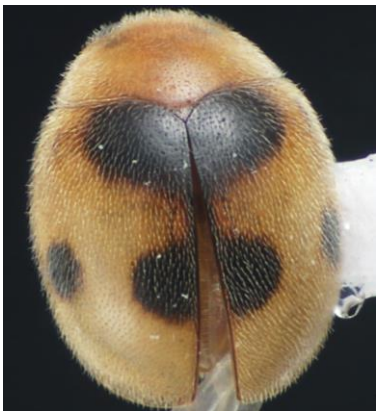
a

b

c

d

e



f

Plate 23. Predators recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

a.*Brumoides suturalis* (10 X), b.*Cheilomenes sexmaculata* (8 X), c.*Cryptolaemus montrouzieri* (8 X), d. *Hyperaspis maindroni* (8 X), e.*Pharoscymnus horni*, (16X) f. *Scymnus coccivora* (20 X)

g

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Plate 23. Predators recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

g. *Scymnus* sp.1 (20X), h. *Scymnus* sp.2 (20X), i. *Scymnus* sp.3 (20X), j. *Scymnus* sp.4 (20X), k. *Scymnus* sp.5 (20X), l. *Scymnus* sp.6 (20X)

m

n

o

p

Plate 23. Predators recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

m. *Nephus* sp. (16 X), n. unidentified sp.1 (16 X), o. unidentified sp. 2 (16 X), p. unidentified sp. 3 (16 X)

q

r

s

t

Plate 23. Predators recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

q. *Spalgis epius*, r. *Diadiplosis* sp. (20X), s. *Cacoxenus* sp (10X), t. *Chrysoperla* sp. (8X)

4.1.8.2 *Parasitoids*

Parasitoids of mealybugs recorded from Kerala during the study is depicted in Table 14.

The study on parasitoids of mealybugs revealed the presence of 11 species belongs to 5 families, in which majority of the species recorded from the family Encyrtidae. Four new host-parasitoid associations were also recorded for the first time. The specimens were identified by Dr. Shahid Bin Zeya and Dr. Mohammad Hayat, Professor, Department of Zoology, Aligarh Muslim University, Aligarh.

The family Encyrtidae contains five species belonging to the subfamily Tetracneminae, of which the parasitoid, *Aenasius arizonensis* (Girault) (Plate 24a), a common parasitoid in Kerala, was recorded from the mealybugs *P. solenopsis* and *F. virgata. Aenasius advena* Compere (Plate 24b), a solitary internal parasitoid, was also found parasitizing on *F. virgata*.

Another parasitoid, *Blepyrus insularis* (Cameron) (Plate 24c), an internal parasitoid, was recorded from mealybugs *F. virgata, P. solenopsis* and *P. jackbeardsleyi*. A new host-parasitoid association is noted with *P. jackbeardsleyi* for the first time.

Two parasitoids belonging to the genera *Leptomastix* namely *L. nigrocincta* (Plate 24d) and *L. tsukumiensis* (Plate 24e) were documented from the mealybug, *C. insolita.* The host-parasitoid association of *C. insolita* with *L. tsukumiensis* is a new record.

The family Eriaporidae was recorded with two parasitoids *viz., Promuscidea unfasciativentris* Girault (Plate 24f) and *Myiocnema comperei* Ashmead (Plate 24g) belong to the subfamilies Eriaporinae and Euryischinae respectively. *P. unfasciativentris* was associated with four species of mealybugs such as *C. insolita, P. marginatus, F. virgata* and *P. solenopsis* whereas *M. comperei* was reported from *P. solenopsis.* Two new host-parasitoid associations are recorded which includes *P. unfasciativentris* - *C. insolita* and *P. unfasciativentris* - *P. marginatus.*

Table 14. Parasitoids recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

Sl. No.	Species	Family	Host insect	Location
1	*Aenasius arizonensis* (Girault)	Encyrtidae: Tetracneminae	*F. virgata*	Vellayani
				Tavanur
			F. virgata	Vellayani
2	*Aenasius advena* Compere	Encyrtidae: Tetracneminae	*F. virgata*	Vellayani
3	*Blepyrus insularis* (Cameron)	Encyrtidae : Tetracneminae	*F. virgata*	Vellayani
				Ambalavayal
			P. solenopsis	Thiruvallom
			*P. jackbeardsleyi**	Karavaram
4	*Leptomastix nigrocincta* Risbec	Encyrtidae: Tetracneminae	*C. insolita*	Amabalathara
5	*Leptomastix tsukumiensis* Tachikawa	Encyrtidae: Tetracneminae	*C. insolita**	Mannakkudi
6	*Promuscidea unfasciativentris* Girault	Eriaporidae: Eriaporinae	*C. insolita* *	Prakandam
			*P. marginatus**	Vellayani
			F. virgata	Vellayani
			P. solenopsis	Vellayani
7	*Myiocnema comperei* Ashmead	Eriaporidae: Euryischinae	*P. solenopsis*	Kayamkulam
8	*unidentified*	Superfamily Proctotrupoidea	*P. jackbeardsleyi*	Ambalavayal
9	*unidentified*	Superfamily Proctotrupoidea	*C. insolita*	Amabalathara
10	*unidentified*	Aphelinidae:	*P. jackbeardsleyi*	Ambalavayal
11	*unidentified*	Pteromalidae	*P. solenopsis*	Kayamkulam

a

b

c

d

e

f

Plate 24. Parasitoids recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

a. *Aenasius arizonensis* (20X), b. *Aenasius advena* (20X), c. *Blepyrus insularis* (20X), d. *Leptomastix nigrocincta* (16X), e. *Leptomastix tsukumiensis* (16X), f. *Promuscidea unfasciativentris* (20 X)

g

h

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j

k

l

Plate 24. Parasitoids recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

g. *Myiocnema comperei* (20X), h. *Superfamily Proctotrupoidea* (25X), i. *Superfamily Proctotrupoidea* (25X), j. *Aphelinidae* (16X), k. *Pteromalidae* (25X), l. Unidentified species (20 X)

Table 15. Hyper parasitoids of mealybugs infesting solanaceous and cucurbitaceous vegetables in different agro ecological units of Kerala

Sl. No	Species	Family	Associated mealybug	Plants surveyed	Location
1	*Cheiloneurus* sp. *	Encyrtidae : Encyrtinae	*P. solenopsis*	*Sesamum indicum*	Kayamkulam
2	*Cheiloneurus* sp.	Encyrtidae : Encyrtinae	*P. solenopsis*	*Cucurbita pepo*	Pattambi
3	*Cheiloneurus* sp.	Encyrtidae : Encyrtinae	*P. solenopsis*	*Sesamum indicum*	Kayamkulam
4	*Cheiloneurus* sp.	Encyrtidae : Encyrtinae	*P. solenopsis*	*Solanum* sp.	Vellayani
5	*Prochiloneurus pulchellus* Silvestri	Encyrtidae : Encyrtinae	*C. insolita*	*Solanum melongena*	Vellayani
6	*Prochilonerus* sp. *	Encyrtidae : Encyrtinae	*P. solenopsis*	*Euphorbia* sp.	Kayamkulam

*new species

Two species of unidentified parasitoids belong to the superfamily Proctotrupoidea (Plate 24h, i) was also recorded from the mealybugs *P. jackbeardsleyi* and *C. insolita.* Two other unidentified parasitoids each from *P. jackbeardsleyi* and, *P. solenopsis* were also reported from Kerala during the study (Plate 24j-l).

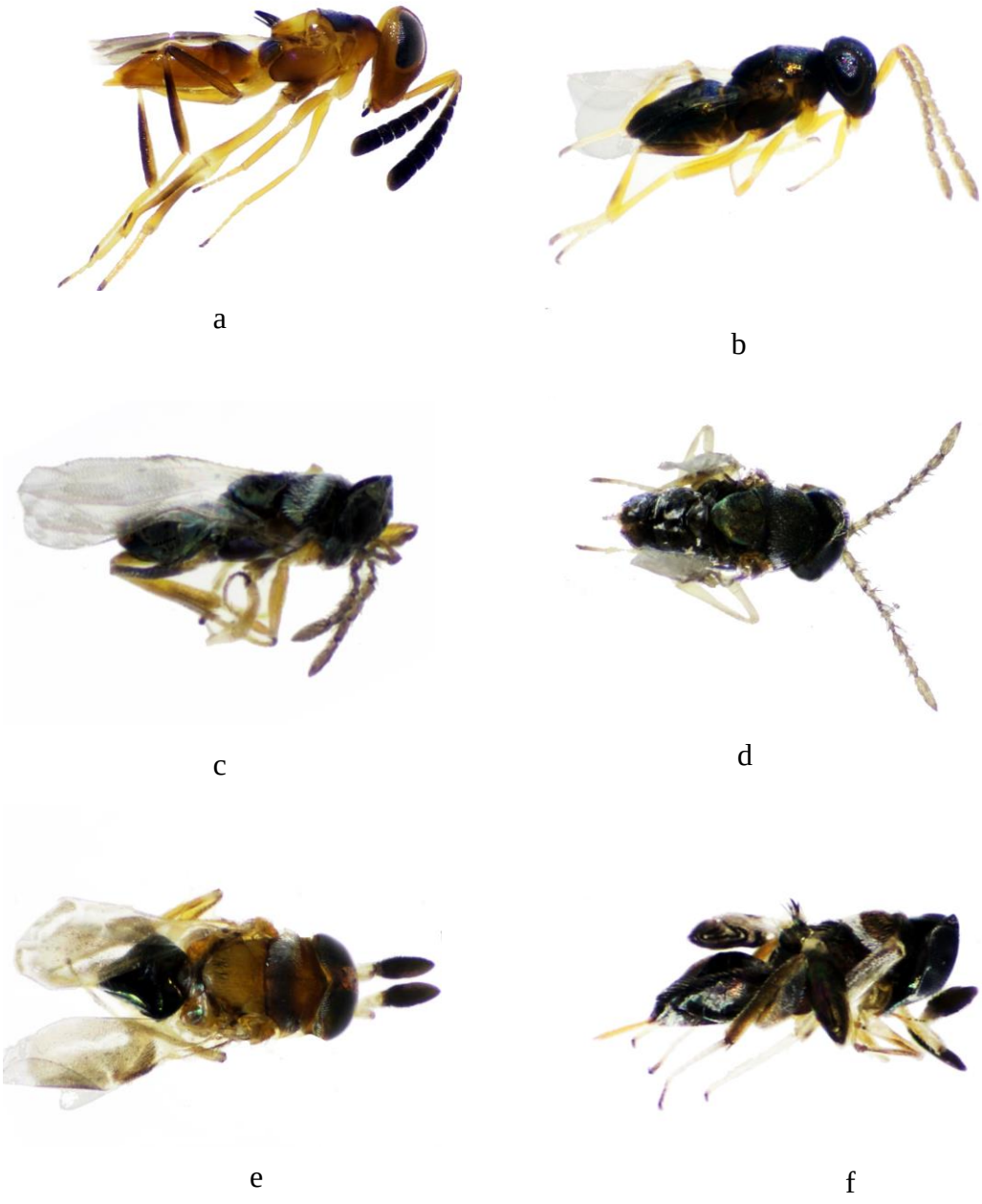
The study also identified six hyperparasitoids (Table 16) belongs to the genera *Cheiloneurus* and *Prochiloneurus* under the family Encyrtidae. Two species were recorded as new report which includes *Cheiloneurus* sp. (Plate 25a) associated with the mealybug *P. solenopsis* in *Sesamum indicum* and *Prochilonerus* sp. (Plate 25 f) associated with the mealybug *P. solenopsis* in *Euphorbia* sp. Besides, three species of *Cheiloneurus* (Plate 25 b, c, d) associated with *P. solenopsis* and another parasitoid *Prochiloneurus pulchellus* Silvestri (Plate 25 e) associated with *C. insolita* was also noted as hyperparasitoids. The specimens were identified by Dr. Shahid Bin Zeya and Dr. Mohammad Hayat, Professor, Department of Zoology, Aligarh Muslim University, Aligarh.

4.1.9 Documentation of Ants Associated with Mealybugs

The list of ants associated with the mealybugs is presented in Table 16.

A total of 14 species of ants belongs to nine genera under three subfamilies were found associated with mealybugs from the study area. The most dominant subfamily observed was Formicinae followed by Myrmicinae and Dolichoderinae. The ant species were identified by Dr. Himender Bharti, Head, Department of Zoology and Environmental Sciences, Ant Systematics and Molecular Biology Lab, Punjab University Patiala, Punjab and Dr. Aniruddha Marathe, Ashoka Trust for Research in Ecology and the Environment (ATREE), Srirampura, Bangalore.

The subfamily Formicinae was recorded with four genera which include *Camponotus, Oecophylla, Anoplolepis* and *Paratrechina.* The maximum species diversity was observed with the genus *Camponotus* which comprises four species



a b c d e f

Plate 25. Hyper parasitoids recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

a.*Cheiloneurus* sp. (20X), b.*Cheiloneurus* sp. (25X), c.*Cheiloneurus* sp. (32X), d.*Cheiloneurus* sp. (25X), e. *Prochiloneurus pulchellus* (20 X), f. *Prochilonerus* sp. (20 X)

viz., C. compressus (Plate 26a), *C. parius* (Plate 26b), *C. barbatus* (Plate 26c) and *C. sericeus* (Plate 26d). The species *C. compressus* was found associated with the mealybugs *C. insolita* and *P. solenopsis* whereas *C. parius* was observed in the colonies of *C. insolita* and *P. citri. C. barbatus* and *C. sericeus* were associated with *P. marginatus*, but *C. barbatus* recorded an additional association with *C. insolita*. The genus *Camponotus* commonly known as carpenter ants, were comparatively large-sized ants with aggressive nature, usually wandering through the mealybug colonies and often feeding on the honeydew of the mealybug.

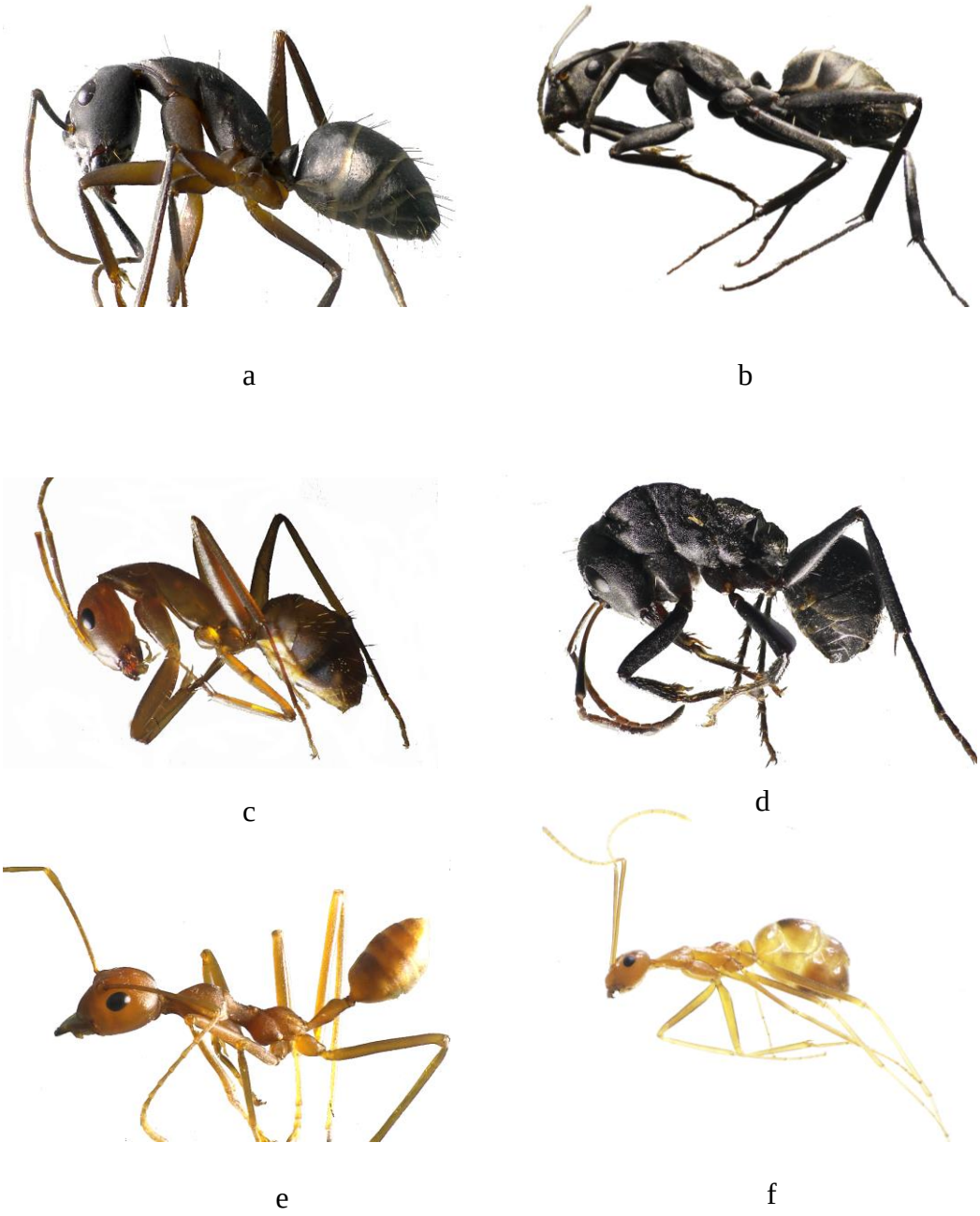
Other genera in the subfamily Formicinae were recorded with a single species each namely *Oecophylla smaragdina* (Plate 26e), *Anoplolepis gracilipes* (Plate 26f) and *Paratrechina longicornis* (Plate 26g). The red weaver ants, *O. smaragdina* was found associated with the mealybugs, *C. insolita* and *P. lilacinus.* These ants were very aggressive and aid in the dispersal of crawlers The ant tended colonies were observed with a low incidence of natural enemies.

Table 16. Ant species associated with mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

Sl. No	Species name	Family	Associated mealy bug species	Plants surveyed	Location
1.	*Camponotus compressus* (Fabricius)	Formicidae: Formicinae	**C. insolita*	*Solanum melongena*	Vellayani
			P. solenopsis	*Solanum torvum*	Vellayani
2.	*Camponotus parius* Emery	Formicidae: Formicinae	**C. insolita*	*Solanum melongena*	Vellayani
			**P. citri*	*Solanum melongena*	Vellayani
3.	*Camponotus barbatus taylori* Forel.	Formicidae: Formicinae	**P. marginatus*	*Abelmoschus esculentus*	Pilicode
			**C. insolita*	*Solanum melongena*	Vellayani
4.	*Camponotus sericeus* (Fabricius)	Formicidae: Formicinae	*P. marginatus*	*Hibiscus mutabilis*	Vellanikkara
5.	*Oecophylla smaragdina* (Fabricius)	Formicidae: Formicinae	**C. insolita*	*Solanum melongena*	Vellayani
			P. lilacinus	*Theobroma cacao* *Vitex negundo* *Polyalthia longifolia*	
6.	*Anoplolepis gracilipes* (Smith, F.)	Formicidae: Formicinae	*C. insolita*	*Solanum melongena*	Vellayani
			**P. marginatus*	*Hibiscus mutabilis*	Vellanikkara
			**P. citri*	*Solanum melongena*	Sadanandapuram
			P. solenopsis	*Capsicum annuum*	Kayamkulam
7.	*Paratrechina longicornis* (Latreille)	Formicidae: Formicinae	**C. insolita*	*Solanum melongena*	Vellayani Tavanur

8.	*Crematogaster subnuda* Mayer	Formicidae: Myrmicinae	**P. citri*	*Canna* sp.	Vellayani
9.	*Solenopsis geminata* (Fabricius)	Formicidae: Myrmicinae	*P. marginatus*	*Solanum melongena*	Vellanikkara
10.	*Solenopsis* sp.	Formicidae: Myrmicinae	*C. insolita*	*Solanum melongena*	Kayamkulam
11.	*Solenopsis globularia* (Smith, F.)	Formicidae: Myrmicinae	**C. insolita*	*Solanum melongena*	Balaramapuram
12.	*Vollenhovia* sp.	Formicidae: Myrmicinae	**P. solenopsis*	*Solanum lycopersicum*	Tavanur
13.	*Tapinoma melanocephalum* (Fabricius)	Formicidae : Dolichoderinae	**C. insolita*	*Solanum melongena*	Kayamkulam
			P. citri	*Solanum melongena*	Kayamkulam
14.	*Technomyrmex albipes* (Smith, F.)	Formicidae: Dolichoderinae	**F. virgata*	*Vigna unguiculata* *Colocasia esculenta*	Anchumukku
			**P. solenopsis*	*Ixora* sp.	Kumarakom

*New mealybug- ant associations recorded in the present study



a b c d e f

Plate 26. Ant species associated with mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala
a. *Camponotus compressus* (8X), b. *Camponotus parius* (8X), c. *Camponotus barbatus* (8X), d. *Camponotus sericeus* (8X), e. *Oecophylla smaragdina* (8X), f. *Anoplolepis gracilipes* (8X)

g

h

i

j

Plate 26. Ant species associated with mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

g. *Paratrechina longicornis* (12X), h. *Solenopsis geminata* (12X), i. *Solenopsis* sp. (16X) , j. *Solenopsis globularia* (16X)

k

l

k. *Crematogaster subnuda* (16X)

m

n

Plate 26. Ant species associated with mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

k. *Crematogaster subnuda* (16X), l. *Vollenhovia* sp. (20 X), m. *Tapinoma melanocephalum* (20X), n. *Technomyrmex albipes* (20X)

a.

b.

c.

d.

e.

f.

g.

h.

i

Plate 27. Ant association with mealybugs infesting solanaceous and cucurbitaceous crops in Kerala.

a. *C. Compressus - C. insolita, b. C. parius - P. citri, c.C. parius - C. insolita, d. C. barbatus-C. insolita, e. C. barbatus-P. marginatus, f. Oecophylla* Sp.*-C. insolita , g. Paratrechina* sp.*- C. insolita h. Anoplolepis* sp.*–C. insolita, i. Anoplolepis* sp.*-P. citri*

j.

k

l.

m.

n.

o.

Plate 27. Ant association with mealybugs infesting solanaceous and cucurbitaceous crops in Kerala.

j. *S. geminata – P. marginatus, k. Vollenhovia sp- P. solenopsis, l. Solenopsis sp.- P. solenopsis, m. Tapinoma* sp.- *C. insolita, n. Tapinoma sp.- P. citri, o. Technomyrmex sp. – P. solenopsis*

This species constructs the nest near mealybug colonies by folding leaves and sometimes mealybug nymphs were observed inside the nest. The yellow crazy ants, *A. gracilipes* was found to be tending four species of mealybugs *viz., C. insolita, P. marginatus, P. citri* and *P. solenopsis.* These ant species was found less aggressive and aggregated near the mealybug colonies. The black crazy ant, *P. longicornis* was found associated with *C. insolita* and were actively tending the mealybug colony.

A total of five species of ants belongs to the genera *Crematogaster*, *Solenopsis* and *Vollenhovia* were recorded from the subfamily Myrmicinae. The genus *Solenopsis* comprised of *S. geminata* (Plate 26h), *Solenopsis* sp. (Plate 26i) and *S. globularia* (Plate 26j). The fire ant, *S. geminata* was recorded from the colony of *P. marginatus* whereas the other two species were recorded from *C. insolita* colony. Being very aggressive, the mealybug colonies tended by fire ants were observed with less incidence of predators and parasitoids compared to untended colonies. The ants *viz., Vollenhovia* sp. (Plate 26l) and *Crematogaster subnuda* (Plate 26 k) were observed in the mealybug colony of *P. solenopsis* and *P. citri* respectively.

The subfamily Dolichoderinae comprised of two species of ants *viz., Tapinoma melanocephalum* (Plate 26m) and *Technomyrmex albipes* (Plate 26n). The ghost ant, *T. melanocephalum* was documented from the colony of mealybug *C. insolita* and *P. citri* while the white footed ant *T. albipes* was found to be associated with *F. virgata* and *P. solenopsis.* . The ant - mealybug associations recorded in Kerala were depicted in Plate 27. The present study revealed 15 new ant-mealybug associations for the first time.

4.2 MOLECULAR CHARACTERIZATION OF MEALYBUGS IN SOLANACEOUS AND CUCURBIRTACEOUS VEGETABLES

The genomic DNA of the mealybug was isolated by QIAGEN DNeasy® blood and tissue kit and the PCR products were sequenced at Rajiv Gandhi Centre for Biotechnology.

Table 17. Homology of sequences generated in the NCBI database

Sl.no	Sample code	Species	Query coverage	Identity percent	E value	Corresponding hits	Corresponding species	Accession number obtained
1	CIV006	*Coccidohystrix insolita*	96	91.26	2e-146	AB439518.1	*Phenacoccus pergandei*	OK036464
2	CHK009	*Crisicoccus hirsutus*	92	95.48	1e-167	MG833841.1	*Planococcus citri*	OK036342
3	FVV 002	*Ferrisia virgata*	94	98.96	0.0	LC278435	*Ferrisia virgata*	OK036484
4	MHK004	*Maconellicoccus hirsutus*	92	99.47	0.0	MG833842.1	*Maconellicoccus hirsutus*	OK036347
5	PSP001	*Phenacoccus solenopsis*	97	99.74	0.0	MF966988.1	*Phenacoccus solenopsis*	OK044467
6	PCV007	*Planococcus citri*	96	98.72	0.0	LC121494.1	*Planococcus minor*	OK043828
7	PLV008	*Planococcus lilacinus*	94	99.23	0.0	MG887768.1	*Planococcus lilacinus*	OK036473
8	PJV003	*Pseudococcus jackbeardsleyi*	95	99.74	0.0	MG940975.1	*Pseudococcus jackbeardsleyi*	OK036466

The nucleotide sequences obtained were checked for sequence similarity using nucleotide BLAST at the NCBI database and also submitted to generate accession number (Table 17).

A total of 21 nucleotide sequences of mealybugs that belongs to five species infesting solanaceous and cucurbitaceous vegetable and three species infesting other vegetable crops were obtained through molecular characterization studies. However, nucleotide sequences of the two species were not obtained. The nucleotide sequences of eight species are given below.

CIV 006- *Coccidohystrix insolita*

CAGAGGAAAAATTTTTATTTTACCTGGATTTGGAGTTATATCTCAAATT
ATAAATCAAGAAAATGGAAAAATAGAAATTTTTAGAAAAATTAATAT
AATTTATGCTATAATTTCAATTGGGATTTTAGGTTTTATTGTTTGAGCC
CATCACATATTTACTATTGGTCTTGATATTGATACTCAACTTTATTTTAT
ATCAGCAACTATAATTATTGCTATCCCAACAAGAATTAAAATTTTTAG
ATGATTAATAACTTTAAATGGAAAAAAAAACAATAAATCATCAATTAA
TTTATGATCAATAGGATTTATTTTAATATTCACTTTAGGAGGATTAACA
GGAATTATTTTATCAAATTCAATTATTGACATTAATTTACATGATACCT
ATTACGTTGTTGCAAG

CHK 007- *Crisicoccus hirsutus*

CTGAAAAAAAATTTTTTATTTTACCAGGATTTGGAATTATATCTCAAAT
TATAAATCAAGAAAGAGGAAAATTAGAAATTTTTAGTAAAATTAATAT
AATTTTTGCAATAATTTCTATTGGAATTTTAGGATTTATTGTCTGAGCC
CATCATATATTTACTATTGGATTAGATATTGACACACAATTATATTTTA
TATCAGCTACAATAATTATTGCTATTCCTACAAGAATTAAAATTTTCAG
ATGAATAATAACTTTAAACGGTAAAAAAATTTTAAATTCATCTATTAA
TTTTTGATCAATAGGATTTATTATTATATTTACTTTAGGCGGTTTAACA
GGAATTATTTTATCTAATTCTATTATTGATATTAATCTTCATGATACCT
ATTACGTAGTTGCCGA

FVV 002- *Ferrisia virgata*

CGGGTTAATTTTTCTTATTTTACCAGGATTTGGTGCTATATCACAAATT
ATAAATCAAGAAACTGGAAAAATTGAAATTTTTAGAAAGATTAATATA
ATTTTTGCTATAATATCAATTGGAATTTTAGGTTTTATTGTTTGAGCTC
ATCATATATTTACTATTGGATTAGATATTGATACACAAATATATTTTAT
AACAGCTACAATAATTATTGCTATTCCAACTAGAATTAAAATTTTTAG
ATGAATAATAACATTAAATGGAAAAAAATTATAAATTCATCAATTACT
TTTTGATCAATTGGATTTATTATTATATTTACACTAGGAGGATTAACTG
GAATTATTTTATCTAATTCAATTATTGATATTAATCTTCATGATACCTA
TTACGTTGTTGCCAGAA

MHK 004-*Maconellicoccus hirsutus*

CCCGGGGTAATATTTTTAATTTTACCAGGATTCGGAGTTATATCTCAAA
TTATGAATCAAGAAAGAGGAAAAATTGAAATCTTTAGAAAAATTAAT
ATAATTTTTGCAATAATTTCTATTGGTATTCTAGGTTTTATTGTTTGAGC
CCATCATATATTCACTATTGGTTTAGATATTGATACACAATTTTATTTT
ATATCAGCTACAATAATTATTGCAATTCCTACAAGAATTAAAATTTTTA
GATGAATAATAACTTTAAATGGAAAAAAAATTTTAAATTCTTCTATTA
ATTATTGATCAATAGGTTTCATTATTATATTTACATTAGGAGGATTAAC
AGGAATCATTTTATCTAATTCTATTATTGATATTAATCTTCATGATACC
TATTACGTAGTTGCCAGC

PSV 005- *Phenacoccus solenopsis*

CAAGCTAAATTTTATTTTACCTGGATTTGGAATTATATCACAAATTATA
AATCAAGAAACAGGAAAAATTGAAATTTTTAGAAAAATTAATATAATT
TATGCTATAATTTCAATTGGAATTTTAGGATTTATTGTTTGAGCTCATC
ATATATTTACTATTGGTTTAGATATTGATACTCAATTATATTTTATATC
AGCAACTATAATTATTGCAATTCCTACAAGAATTAAAATTTTTAGATG
ATTAATAACACTTAATGGAAAAAAAATATTTAATTCATCAACTAATTT
TTGATCTATTGGATTTATTATTATATTTACTTTAGGGGGATTAACTGGT

ATTATTCTTTCAAACTCTATTATTGATATTAACTTACATGATACCTATT
ACGTAGTTGCCAA

PCV 007- *Planococcus citri*

CAGCTTTAAATTTTATTTTACCAGGTTTTGGAACTATATCCCAAATTAT
AAATCAAGAAAGAGGAAAAATAGAAATTTTTAGTAAAATTAATATAA
TTTTTGCTATAATTTCAATTGGAATTTTAGGTTTTATTGTTTGAGCTCAT
CATATATTTACTATCGGATTAGATATTGATACACAATTATATTTTATAT
CAGCTACAATAATTATTGCTATCCCTACAAGAATTAAAATCTTTAGAT
GAATAATAACTTTAAATGGTAAAAAAATTCTTAATTCATCTATTAACTT
TTGATCAATTGGATTCATCATTATATTTACATTAGGAGGATTAACTGGA
ATTATTTTATCAAATTCTATTATTGATATTAATCTTCATGATACCTATTA
CGTAGTTGCAAGA

PLV005- *Planococcus lilacinus*

CGGAGGGTTTAATTTTTAAATTTTACCAGGATTTGGAATAATATCTCAA
ATTATAAACCAAGAAAGAGGAAAAATAGAAATTTTTAGTAAAATTAA
TATAATTTTTGCAATAATTTCCATTGGAATTTTAGGTTTTATTGTTTGAG
CTCATCATATATTTACTATTGGATTAGACATTGACACTCAATTATATTT
TATATCAGCTACAATAATTATTGCTATCCCTACTAGAATTAAAATTTTC
AGATGAATAATAACTTTAAATGGAAAAAAAATTTTAAATTCATCAATT
AATTTTTGATCAATTGGATTTATTATTATATTTACTTTAGGGGGTTTAA
CAGGTATCATTTTATCTAATTCAATTATTGATATTAACTTACATGATAC
CTATTACGTAGTTGCCAAA

PJV 003- *Pseudococcus jackbeardsleyi*

CCTAAGGTATTTTTATTTTACCAGGTTTTGGTTTAATATCACAAATTAT
AAATCAAGAAAGAGGAAAATTAGAAATTTTTAGAAAAATAAATATAA
TTTTTGCAATAATTTCAATTGGAATTTTAGGTTTTATTGTATGAGCTCA
TCATATATTTACTATTGGTTTAGATATTGATACTCAAATATATTTTATA
TCAGCTACAATAATTATTGCTATTCCAACTAGTATTAAAATTTTTAGAT
GAATAATAACACTTAATGGAAAAAAAAAATCTAATTCATCTATTTTAA

TATGATCAATAGGATTTATTATTATATTTACCATAGGAGGATTAACAG
GAATTATTTTATCAAATTCAATTATTGACGTCAATTTACATGATACCTA
TTACGTAGTAGCAA

Among the eight mealybug species, four species *viz., P. solenopsis, M. hirsutus, P. jackbeardsleyi* and *P. lilacinus* showed more than 99 % similarity with the existing sequences in NCBI-Gen Bank whereas the species *F. virgata* showed 98.96 % similarity. However, the species identified as *P. citri* through taxonomic identification exhibited more similarity to the sequence of *P. minor* in the NCBI database. The species *C. hirsutus* exhibited 95.48 % similarity to the sequence submitted as *P. citri* in the database and 94.24 % similarity to *C. hirsutus* accession. The species *C. insolita* did not exhibit much similarity with the available accessions in NCBI database and it showed only 91.26 % similarity to *Phenacoccus pergandei* Cockerell.

A total of nine sequences of mealybugs were submitted to the Barcode Of Life Data systems (BOLD) to generate the DNA barcodes. The process ID obtained for the sequences are represented in Table 18. The illustrative DNA barcodes are also portrayed for eight sequences (Fig. 6).

4.3 MOLECULAR CHARACTERIZATION OF GUT ENDOSYMBIONTS OF *C. INSOLITA*

A thorough knowledge on the diversity of endosymbionts aid in better understanding of species interactions in the ecosystem. The study on gut endosymbionts of the mealybug, *C. insolita* was conducted for the first time.

The metagenomic DNA was isolated from the mealybug, *C. insolita* and the quality and quantity of DNA was evaluated on Nanodrop by determining A_{260}/A_{280} value. The metagenomic DNA was quantified as 208.2 ng/µl with A_{260}/A_{280} ratio of 1.87 which indicated that the DNA was of good quality. The amplicon was generated using the metagenomic DNA along with the bacterial 16S V3-V4 region-specific primer and a clear band was observed when resolved on 1.2 % Agarose gel.

Table 18. Process ID of the mealybug species submitted to Barcode Of Life Database

Sl.no	Sample code	Process ID	Name of Species
1	CIV006	MITRA001-21	*Coccidohystrix insolita*
2	CHK007	MEALY005-21	*Crisicoccus hirsutus*
3	FVV 002	MEALY004-21	*Ferrisia virgata*
4	MHK004	MEALY006-21	*Maconellicoccus hirsutus*
5	PSP001	MEALY007-21	*Phenacoccus solenopsis*
6	PCV007	MEALY008-21	*Planococcus citri*
7	PLV005	MEALY002-21	*Planococcus lilacinus*
8	PJV003	MEALY001-21	*Pseudococcus jackbeardsleyi*

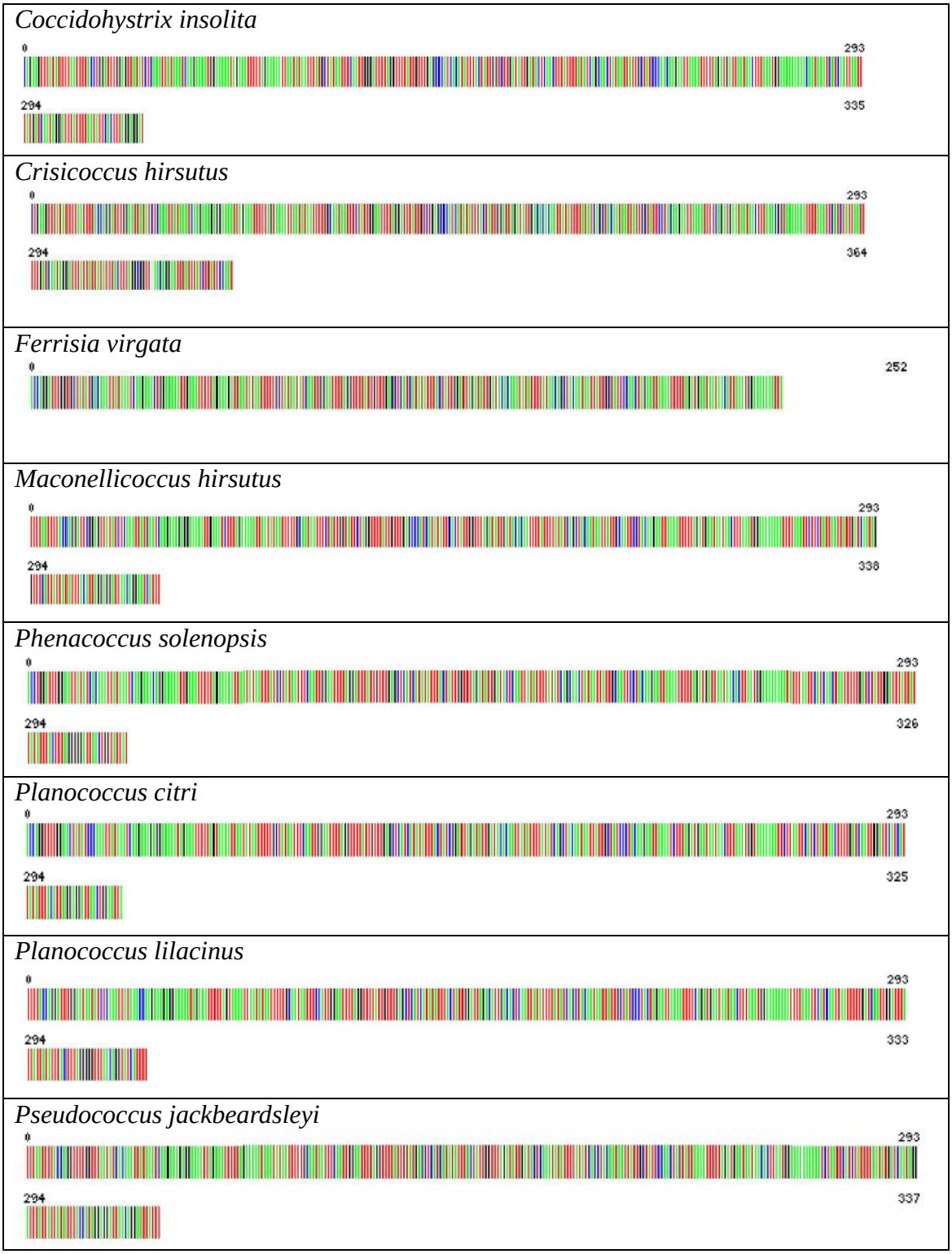


Fig 6. Illustrative barcode of different mealybug species submitted in Barcode Of Life Database BOLD)

A standard protocol was followed for 16S rRNA library preparation and the sample was loaded to the Illumina MiSeqTM sequencer. A total of 239,427 high quality reads were obtained and were pooled and processed to 381 operational taxonomic units (OTU).

The endosymbionts recorded from the mealybug, *C. insolita* were analyzed and classified into each taxonomic category from phyla to species level.

A total of 15 phyla of endosymbionts belongs to two kingdoms *viz.*, Bacteria and Archaea were recorded from the mealybug *C. insolita.* The kingdom Bacteria consist of 13 phyla, of which the phylum Proteobacteria was the dominant one with 60.37 per cent of the total endosymbiont population. The second dominant phylum was Euryarchaeota belongs to the kingdom Archaea which contributes about 23.88 per cent of total endosymbiont population. It was followed by the bacteria belong to the phyla Firmicutes (8.55%), Bacteroidetes (3.81%), Actinobacteria (1.24%), Acidobacteria (0.96%), Planomycetes (0.68%), Spirochaetes (0.2%), Verrucomicrobia (0.17%) and Thermi (0.02%). The phylum Crenarcheota belongs to the kingdom Archaea accounts for a minor share of 0.02 per cent population of endosymbionts. Similarly, the phyla Tenericutes, Chloroflexi and Fusobacteria were also recorded as the endosymbionts of the mealybug, *C. insolita* with a very negligible population abundance of 0.01per cent (Fig.7).

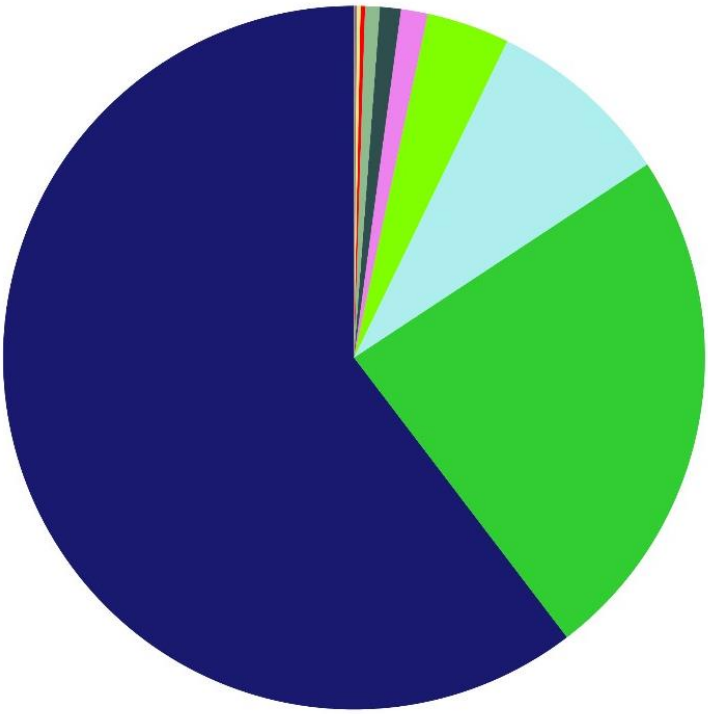
The comparison on the relative abundance of endosymbionts of mealybugs at class level revealed a total of 19 classes of bacteria, of which Gammaproteobacteria was recorded as the most abundant group (41.36%). It was followed by Halobacteria (23.83%), Alphaproteobacteria (11.66%), Bacilli (7.58%), Betaproteobacteria (7.34%), Bacteroidia (2.95%) and Actinobacteria (1.18%). The class Clostridia accounts for 0.86 per cent population of total endosymbionts which was followed by Acidobacteria (0.8%), Planctomycetia (0.67%), Saprospirae (0.42%), Flavobacteria (0.39%) and Spirochaetes (0.2%). The remaining one per cent population of endosymbionts consist of Verrucomicrobia (0.16%), Soilbacters (0.15%), Erysipelotrichi (0.11%),

Deinococci (0.07%), Methanobacteria (0.05%), Acidimicrobiia (0.05%) and others (0.18%) (Fig.8)

The categorization of endosymbionts to order level revealed a total of 19 orders, of which the most abundant order was Pseudomonadales (40.55%) followed by Halobacteriales (23.83%) and Sphingomonadales (7.32%). The order Lactobacillales accounts for 6.89 per cent share of total endosymbiont population which was followed by Burkholderiales (4.09%), Tremblayales (3.25%), Bacteroidales (2.95%), Caulobacterales (2.13%), Rhizobiales (1.8%), Actinomycetales (0.92%), Clostridiales (0.86%), Acidobacteriales (0.8%), Bacillales (0.69%), Gemmatales (0.65%), Enterobacteriales (0.62%), Saprospirales (0.42%), Flavobacteriales (0.39%), Bifidobacteriales (0.26%), Spirochaetales (0.2%) and others (1.38%) (Fig.9)

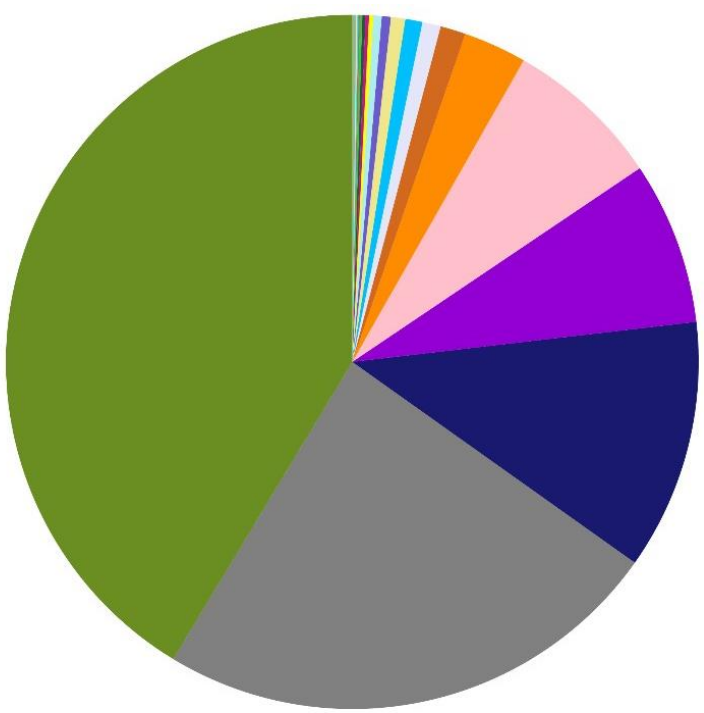
The taxonomic distribution and abundance of endosymbionts of the mealybug *C. insolita* at the family level were analysed and the results showed that a total of 19 families of bacteria were present in the gut of the mealybug. Among the various families, Pseudomonadaceae was recorded with the highest abundance of endosymbionts which was followed by Halobacteriaceae (23.83%) and Sphingomonadaceae (7.25%). The family Lactobacillaceae accounts for 5.89 per cent of gut microbial population which was followed by the Tremblayaceae (3.25%), Alcaligenaceae (2.45%), Prevotellaceae (2.23%), Caulobacteraceae (2.13%), Oxalobacteraceae (1.62%) and Bradyrhizobiaceae (1.07%). The families *viz.,* Acidobacteriaceae Leuconostocaceae, Isosphaeraceae, Enterobacteriaceae, Moraxellaceae, Paraprevotellaceae, Methylobacteriaceae, Chitinophagaceae and Staphylococcaceae were recorded with a share of 0.73 per cent, 0.69 per cent, 0.65 per cent, 0.62 per cent, 0.55 per cent, 0.49 per cent, 0.45 per cent, 0.42 per cent, and 0.42 per cent of total population of microbes respectively (Fig.10).

The genus level distribution of endosymbionts was also studied and the results showed that a total of 19 genera of bacteria were present, in which the genus *Pseudomonas* was recorded as the most dominant one with a share of 39.44 per cent. The second abundant genus was *Halorhabdus* (8.93%), followed by



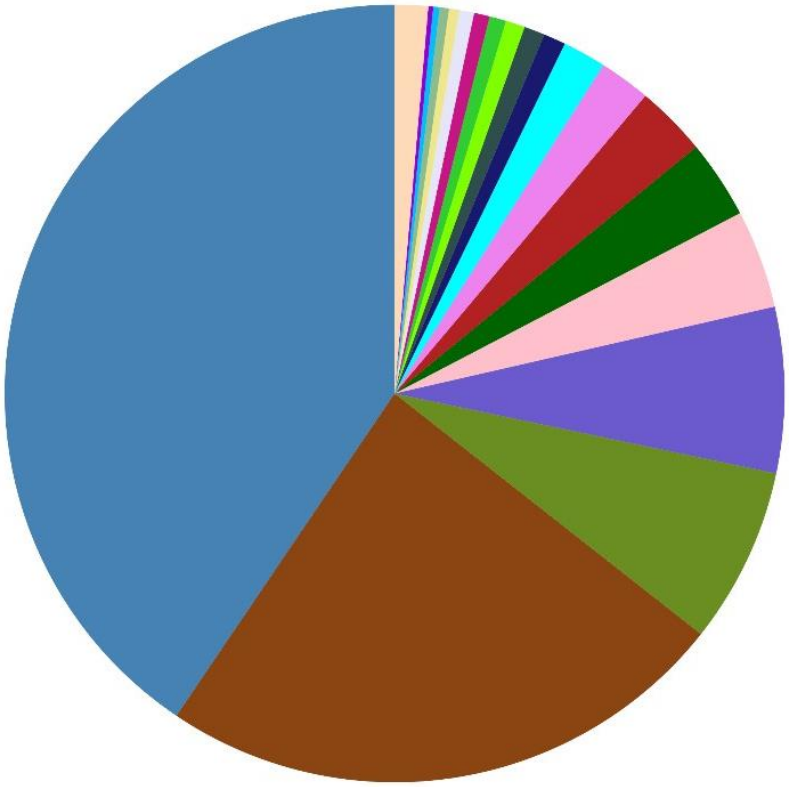
Legends	Taxonomy	Abundance
	k__Bacteria;p__Proteobacteria	60.37%
	k__Archaea;p__Euryarchaeota	23.88%
	k__Bacteria;p__Firmicutes	8.55%
	k__Bacteria;p__Bacteroidetes	3.81%
	k__Bacteria;p__Actinobacteria	1.24%
	k__Bacteria;p__Acidobacteria	0.96%
	k__Bacteria;p__Planctomycetes	0.68%
	k__Bacteria;p__Spirochaetes	0.2%
	k__Bacteria;p__Verrucomicrobia	0.17%
	k__Bacteria;p__[Thermi]	0.07%
	k__Archaea;p__Crenarchaeota	0.02%
	k__Bacteria;p__OD1	0.01%
	k__Bacteria;p__Tenericutes	0.01%
	k__Bacteria;p__Chloroflexi	0.01%
	k__Bacteria;p__Fusobacteria	0.01%

Fig. 7. Taxonomic distribution and abundance of gut microbial community of *Coccidohystrix insolita* - phylum level



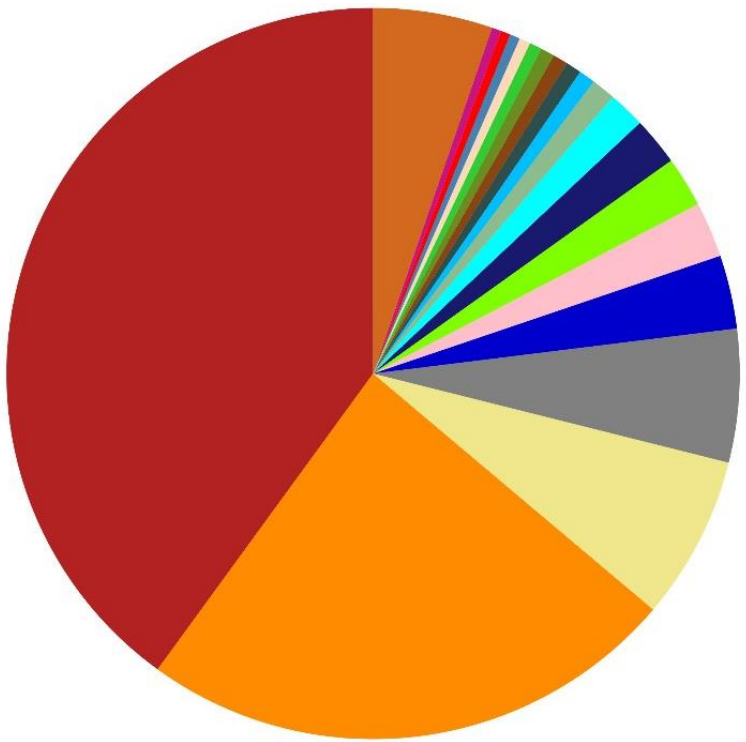
Legends	Taxonomy	Abundance
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	41.36%
	k__Archaea;p__Euryarchaeota;c__Halobacteria	23.83%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	11.66%
	k__Bacteria;p__Firmicutes;c__Bacilli	7.58%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	7.34%
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia	2.95%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria	1.18%
	k__Bacteria;p__Firmicutes;c__Clostridia	0.86%
	k__Bacteria;p__Acidobacteria;c__Acidobacteriia	0.8%
	k__Bacteria;p__Planctomycetes;c__Planctomycetia	0.67%
	k__Bacteria;p__Bacteroidetes;c__[Saprospirae]	0.42%
	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia	0.39%
	k__Bacteria;p__Spirochaetes;c__Spirochaetes	0.2%
	k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae	0.16%
	k__Bacteria;p__Acidobacteria;c__Solibacteres	0.15%
	k__Bacteria;p__Firmicutes;c__Erysipelotrichi	0.11%
	k__Bacteria;p__[Thermi];c__Deinococci	0.07%
	k__Archaea;p__Euryarchaeota;c__Methanobacteria	0.05%
	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia	0.05%
	Others	0.18%

Fig. 8. Taxonomic distribution and abundance of gut microbial community of *Coccidohystrix insolita* - class level



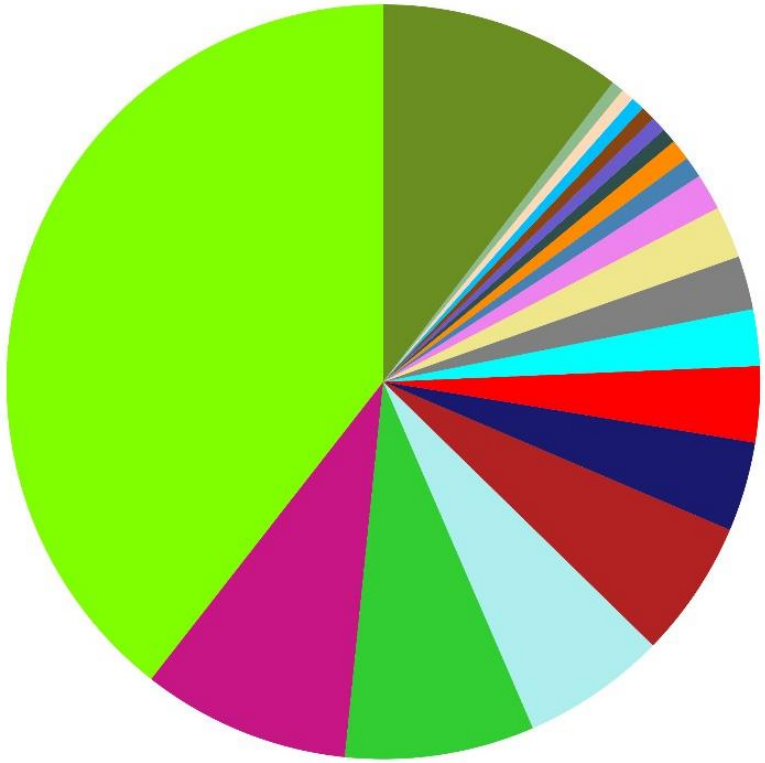
Legends	Taxonomy	Abundance
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales	40.55%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales	23.83%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales	7.32%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales	6.89%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales	4.09%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Tremblayales	3.25%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales	2.95%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales	2.13%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales	1.8%
	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales	0.92%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales	0.86%
	k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales	0.8%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales	0.69%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Gemmatales	0.65%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales	0.62%
	k_Bacteria;p_Bacteroidetes;c_[Saprospirae];o_[Saprospirales]	0.42%
	k_Bacteria;p_Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales	0.39%
	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales	0.26%
	k_Bacteria;p_Spirochaetes;c_Spirochaetes;o_Spirochaetales	0.2%
	Others	1.38%

Fig. 9. Taxonomic distribution and abundance of gut microbial community of *Coccidohystrix insolita*- order level



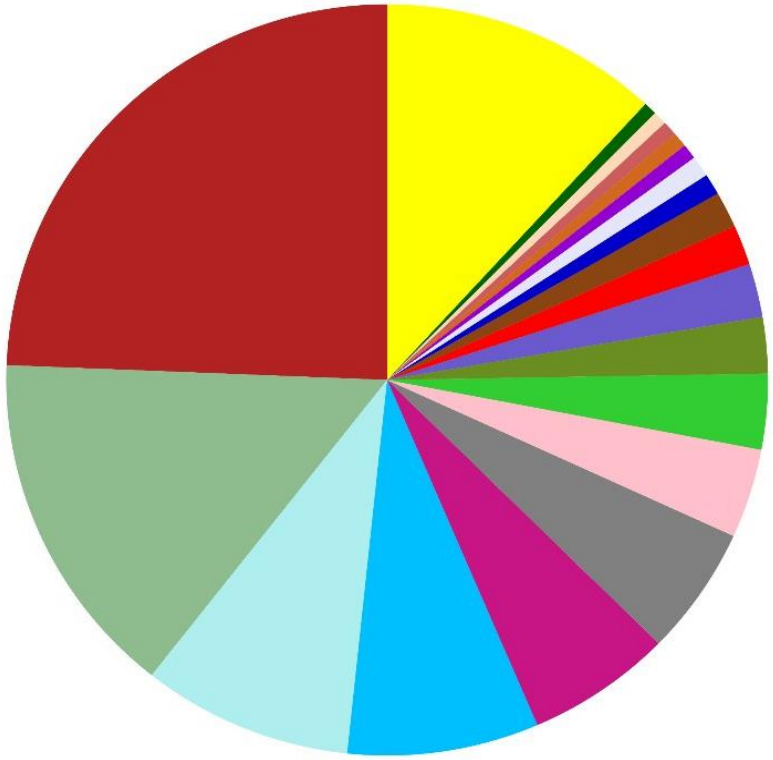
Legends	Taxonomy	Abundance
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae	40.0%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae	23.83%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales;f_Sphingomonadaceae	7.25%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae	5.89%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Tremblayales;f_Tremblayaceae	3.25%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae	2.45%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae	2.23%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteraceae	2.13%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Oxalobacteraceae	1.62%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Bradyrhizobiaceae	1.07%
	k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Acidobacteriaceae	0.73%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Leuconostocaceae	0.69%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Gemmatales;f_Isosphaeraceae	0.65%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae	0.62%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae	0.55%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Paraprevotellaceae]	0.49%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Methylobacteriaceae	0.45%
	k_Bacteria;p_Bacteroidetes;c_[Saprospirae];o_[Saprospirales];f_Chitinophagaceae	0.42%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Staphylococcaceae	0.42%
	Others	5.26%

Fig. 10.Taxonomic distribution and abundance of gut microbial community of *Coccidohystrix insolita* - family level



Legends	Taxonomy	Abundance
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Pseudomonas	39.44%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Halorhabdus	8.93%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Natronococcus	8.13%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales;f_Sphingomonadaceae;g_Novosphingobium	6.19%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus	5.89%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Natronomonas	3.84%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Tremblayales;f_Tremblayaceae;g_Candidatus Tremblaya	3.25%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Achromobacter	2.42%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Unclassified	2.3%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella	2.23%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Oxalobacteraceae;g_Ralstonia	1.56%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteraceae;g_Phenylobacterium	0.91%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteraceae;g_Unclassified	0.9%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Leuconostocaceae;g_Leuconostoc	0.65%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Gemmatales;f_Isosphaeraceae;g_Unclassified	0.65%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales;f_Sphingomonadaceae;g_Unclassified	0.62%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Bradyrhizobiaceae;g_Unclassified	0.58%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Unclassified	0.56%
	k_Bacteria;p_Acidobacteria;c_Acidobacteriia;o_Acidobacteriales;f_Acidobacteriaceae;g_Unclassified	0.53%
	Others	10.42%

Fig. 11. Taxonomic distribution and abundance of gut microbial community of *Coccidohystrix insolita* - genus level



Legends	Taxonomy	Abundance
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Pseudomonas;s_alcaligenes	24.37%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Pseudomonas;s_Unclassified	15.02%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Halorhabdus;s_Unclassified	8.93%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Natronococcus;s_Unclassified	8.13%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales;f_Sphingomonadaceae;g_Novosphingobium;s_Unclassified	6.17%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae;g_Lactobacillus;s_Unclassified	5.55%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Natronomonas;s_Unclassified	3.84%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Tremblayales;f_Tremblayaceae;g_Candidatus Tremblaya;s_Unclassified	3.25%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae;g_Achromobacter;s_Unclassified	2.42%
	k_Archaea;p_Euryarchaeota;c_Halobacteria;o_Halobacteriales;f_Halobacteriaceae;g_Unclassified;s_Unclassified	2.3%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae;g_Prevotella;s_copri	1.72%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Oxalobacteraceae;g_Ralstonia;s_Unclassified	1.56%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteraceae;g_Phenylobacterium;s_Unclassified	0.91%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteraceae;g_Unclassified;s_Unclassified	0.9%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Leuconostocaceae;g_Leuconostoc;s_Unclassified	0.65%
	k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Gemmatales;f_Isosphaeraceae;g_Unclassified;s_Unclassified	0.65%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales;f_Sphingomonadaceae;g_Unclassified;s_Unclassified	0.62%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Bradyrhizobiaceae;g_Unclassified;s_Unclassified	0.58%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Unclassified;s_Unclassified	0.56%
	Others	11.86%

Fig. 12. Taxonomic distribution and abundance of gut microbial community of *Coccidohystrix insolita* - species level

Table 19. Abundance of 10 bacterial species from phyla to species occur in the gut of *Coccidohystrix insolita.*

Sl. No	Phylum	Class	Order	Family	Genus	Species
1	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonus*	*Pseudomonas alcaligenes* (24.37%)
2	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonus*	Unclassified *Pseudomonus* (15.02%)
3	Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	*Halorhabdus*	Unclassified *Halorhabdus* (8.93%)
4	Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	*Natronococcus*	Unclassified *Natronococcus* (8.13%)
5	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingoblum*	Unclassified *Novosphingoblum* (6.17%)
6	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	Unclassified *Lactobacillus* (5.55%)
7	Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	*Natronomonas*	Unclassified *Natronomonas* (3.84%)
8	Proteobacteria	Betaproteobacteria	Tremblayales	Tremblayaceae	*Candidatus Tremblaya*	Unclassified *Candidatus Tremblaya* (3.25%)
9	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Acromobacter*	Unclassified *Achromobacter* (2.42%)
10	Euryarchaeota	Halobacteria	Halobacteriales	Halobacteriaceae	Unclassified	Unclassified (2.3%)

Natronococcus (8.13 %), *Novosphingobium* (6.19%), *Lactobacillus* (5.89%) and *Natronomonas* (3.84%). The genus *Candidatus tremblaya* was recorded with a per cent share of 3.25 which was followed by *Achromobacter* (2.42%), unclassified genus belongs to Halobacteriaceae (2.3%), *Prevotella* (2.23%), *Ralstonia* (1.56%), *Phenylobacterium* (0.91%) and *Leuconosotoc* (0.65%). A total of six genera of unclassified organisms and others (10.42%) contributed to the remaining population of gut endosymbionts of the mealybug, *C. insolita* (Fig.11).

A total of 19 species of gut endosymbionts were recorded from the mealybug, *C. insolita.* The most abundant species was recorded as *Pseudomonas alcaligenes* (24.37%) followed by an unclassified species belong to the genus *Pseudomonas* (15.02%). Unclassified species belongs to the genera *Halorhabdus* (8.93%), *Natronococcus* (8.13%), *Novosphingoblum* (6.17%), *Lactobacillus* (5.55%), *Natronomonas* (3.84%), *Candidatus tremblaya* (3.25%), *Achromobacter* (2.42%) and *Prevotella copri* (1.72%) were also recorded as the major endosymbionts of the mealybug, *C. insolita* (Fig.12) (Table 19).

4.4. TRI-TROPHIC INTERACTION

4.4.1. Interaction in Brinjal Ecosystem Mediated by Mealybug, *C. insolita* and Its Natural Enemies.

A field experiment was conducted at Instructional Farm, Vellayani to identify the tri-trophic interaction in brinjal ecosystem mediated by mealybug, *C. insolita* and its natural enemies.

4.4.1.1. *Estimation of Mealybug Population in Brinjal Cultivars*

The brinjal cultivars tested for their field tolerance to brinjal mealybug, *C. insolita* exhibited significant differences in the population of mealybugs at 65, 75, 85 and 95 days after transplanting. The data on the mean population of mealybugs in different brinjal cultivars is depicted in Table 20.

The mean population of mealybugs at 65 days after transplanting exhibited a significant difference among ten brinjal cultivars screened for field tolerance to *C. insolita* with values ranged from 38.33 to 141.33. Among the ten brinjal

cultivars, the lowest population of mealybugs was recorded in Pusa Uttam (38.33) which was statistically on par with Pusa Purple Long (45.67) and Pusa Kausal (48.00) whereas the highest population was recorded in the cultivar Udit (141.33) which was statistically on par with Pusa Shymala (125.00), Pink Long (125.33), Haritha (124.67), Green Long (116.00) and Neelima (114.67).

At 75 days after transplanting, the mean population of mealybugs varied from 49.67 to 154.33 and were differed significantly among the different cultivars. The lowest population of mealybugs was recorded from Pusa Uttam (49.67) which was on par with Pusa Purple Long (50.33) and Pusa Kausal (63.00). It was followed by the cultivar Ponni with a mean population of 72.67 mealybugs/ three leaves. The highest population of mealybugs was observed in the cultivar Pink Long (154.33) which was statistically on par with Haritha (144.33). It was followed by Udit (123.67) which was statistically on par with Green Long (119.00), Pusa Shymala (115.00) and Neelima (104.33).

The brinjal cultivars differed significantly in the mean population of mealybugs at 85 days after transplanting and it ranged from 41.67 to 148.67. The cultivar Pusa Uttam was found to be less attractive to mealybugs with a mean population of 41.67 mealybugs/ three leaves and was statistically on par with the Pusa Purple Long (45.33). It was followed by Ponni and Pusa Kaushal with a mean population of 60.67 and 64.67 respectively. The highest mean population of mealybugs was harbored by the cultivar Udit (148.67) which was significantly superior to all other cultivars. The cultivar, Pink Long was infested with the second-highest population of mealybugs (116.00) which was statistically on par with Haritha (112.33), Pusa Shymala (108.33), Green Long (107.67) and Neelima (96.33).

The mean population of mealybugs differed significantly among the cultivars and varied from 24.67 to 96.67 at 95 days after transplanting. The lowest mean population of mealybugs was recorded from the cultivar Pusa Uttam (24.67) which was statistically on par with Pusa Purple Long (25.33), Ponni (32.67) and Pusa Kaushal (34.33). The highest mean population of mealybugs was recorded

Table 20. Population of *Coccidohystrix insolita* in different cultivars of brinjal

Cultivars	Mean number of mealybugs/three leaves			
	65 DAT	75 DAT	85 DAT	95 DAT
Haritha	124.67 (11.15)^{a}	144.33 (12.01)^{ab}	112.33 (10.59)^{b}	80.67 (8.94)^{a}
Neelima	114.67 (10.71)^{a}	104.33 (10.20)^{c}	96.33 (9.82)^{b}	52.33 (7.12)^{bc}
Ponni	79.00 (8.73)^{b}	72.67 (8.51)^{d}	60.67 (7.76)^{cd}	32.67 (5.67)^{cd}
Pink Long	125.33 (11.18)^{a}	154.33 (12.41)^{a}	116.00 (10.76)^{b}	71.33 (8.37)^{ab}
Udit	141.33 (11.88)^{a}	123.67 (11.12)^{bc}	148.67 (12.19)^{a}	96.67 (9.81)^{a}
Green Long	116.00 (10.76)^{a}	119.00 (10.91)^{c}	107.67 (10.36)^{b}	47.67 (6.90)^{bc}
Pusa Purple Long	45.67 (6.64)^{c}	50.33 (7.09)^{e}	45.33 (6.71)^{de}	25.33 (5.00)^{d}
Pusa Kaushal	48.00 (6.91)^{c}	63.00 (7.91)^{de}	64.67 (8.02)^{c}	34.33 (5.83)^{cd}
Pusa Uttam	38.33 (6.18)^{c}	49.67 (7.04)^{e}	41.67 (6.42)^{e}	24.67 (4.92)^{d}
Pusa Shyamla	125.00 (11.17)^{a}	115.00 (10.71)^{c}	108.33 (10.38)^{b}	80.67 (8.98)^{a}
SE m±	0.70	0.63	0.61	0.56
CD(0.05)	1.600	0.920	1.234	1.689

*DAT –Days After Transplanting

**Figures in parentheses are √x+0.5 transformed values

*** Means followed by the same letter(s) not significantly different

from Udit (96.67) which was statistically on par with Pusa Shymala (80.67), Haritha (80.67) and Pink Long (71.33). It was followed by Neelima and Green Long which were statistically on par with each other with a mean population of 52.33 and 47.67 mealybugs/ three leaves respectively.

4.4.1.2. *Assessment of Leaf Damage Caused by Mealybug*

The leaf damage caused by *C. insolita* in different brinjal cultivars at 65, 75, 85 and 95 days after transplanting was significantly differed and is presented in Table 21.

Among the tested varieties, the per cent leaf damage varied from 6.49 to 58.96 at 65 days after transplanting. The lowest leaf damage was recorded in Pusa Purple Long (6.49 %) which was statistically on par with Pusa Uttam (10.52 %) and Ponni (11.65 %). The hybrid cultivar Udit was severely damaged by the mealybug with a mean per cent damage of 58.96 which was statistically superior to all other cultivars. The cultivar, Haritha recorded the second highest mean per cent leaf damage of 30.57 which was statistically on par with Pink Long (29.26), Green Long (27.83), Neelima (23.70) and Pusa Shymala (22.24).

At 75 days after transplanting, the leaf damage varied from 9.88 to 67.76 per cent. The cultivar Pusa Purple Long exhibited tolerance to mealybug with a mean per cent leaf damage of 9.88 which was statistically on par with Pusa Uttam (11.72) and Ponni (17.46). The highest leaf damage was observed in the cultivar, Udit (67.76%) which was statistically on par with the cultivar Haritha (60.88%). It was followed by Pink Long, Green Long and Pusa Shymala which were statistically on par with a mean per cent leaf damage of 39.68, 37.62 and 30.94 respectively.

The mean per cent leaf damage caused by mealybug *C. insolita* in brinjal cultivars ranged from 10.41 to 69.62 at 85 days after transplanting. The cultivar Pusa Purple Long exhibited the lowest leaf damage of 10.41 per cent which was statistically on par with Pusa Uttam (13.75%). The cultivar, Udit recorded the highest per cent leaf damage (69.62) which was statistically superior to all other

Table 21. Leaf damage caused by *Coccidohystrix insolita* in different cultivars of brinjal

Cultivars	Leaf damage (%)			
	65 DAT	75 DAT	85 DAT	95 DAT
Haritha	30.57^{b}	60.88^{a}	56.27^{b}	53.17^{a}
Neelima	23.70^{bc}	29.73^{cd}	33.15^{cde}	29.17 ^{b}
Ponni	11.65^{de}	17.46^{ef}	18.89^{f}	20.18^{c}
Pink Long	29.26 ^{b}	39.68^{b}	38.09^{c}	32.19^{b}
Udit	58.96^{a}	67.76^{a}	69.62^{a}	61.18^{a}
Green Long	27.83^{b}	37.62^{bc}	34.81^{cd}	29.72^{b}
Pusa Purple Long	6.49^{e}	9.88^{f}	10.41^{g}	9.08^{d}
Pusa Kaushal	19.03^{cd}	21.44^{de}	26.12 e	34.07^{b}
Pusa Uttam	10.52^{e}	11.72^{f}	13.75^{fg}	17.01^{cd}
Pusa Shyamla	22.24^{bc}	30.94^{bc}	28.47^{de}	32.96^{b}
SE m±	4.69	6.17	5.83	4.95
CD(0.05)	8.423	9.442	7.114	8.142

*DAT-Days After Transplanting

** Means followed by the same letter(s) not significantly different

cultivars. The second-highest mean per cent leaf damage was recorded in Haritha (56.27), which was statistically different from other cultivars. It was followed by Pink Long (38.09) which was statistically on par with Green Long (34.81) and Neelima (33.15). The cultivar Pusa Shymala recorded a mean per cent leaf damage of 28.47 which was statistically on par with Pusa Kaushal (26.12).

The tested brinjal cultivars exhibited significant differences in leaf damage at 95 days after transplanting that ranged from 9.08 to 61.18 per cent. The lowest mean per cent leaf damage was recorded with Pusa Purple Long (9.08) which was statistically on par with Pusa Uttam (17.01). The highest leaf damage was observed in Udit (61.18 %) which was statistically on par with the cultivar, Haritha (53.17 %). The cultivar, Pusa Kaushal exhibited a mean per cent leaf damage of 34.07 which was statistically on par with Pusa Shymala (32.96), Pink Long (32.19), Green Long (29.72) and Neelima (29.17).

4.4.1.3. *Categorization of Cultivars Based on Leaf Damage*

Ten brinjal cultivars were categorized into different groups based on leaf damage caused by *C. insolita* (Table 22). None of the genotype was found to be immune to brinjal mealybug *C. insolita.*

The cultivar, Pusa Purple Long was classified as resistant with a mean per cent leaf damage of 8.96 with a damage score 1. The cultivars *viz.*, Pusa Uttam and Ponni were contained in the group moderately resistant with mean per cent leaf damage of 13.25 and 17.05 respectively with a damage score of 2. Three cultivars were included under the category moderately susceptible with a damage score of 3 which consist of Neelima, Pusa Shymala and Pusa Kaushal with leaf damage of 28.94 28.65 and 25.16 per cent respectively. The susceptible category consists of hybrid cultivars *viz.*, Pink Long and Green Long with a mean per cent leaf damage of 34.80 and 32.49 respectively and a damage score of 4. Udit and Haritha were included under highly susceptible category based on the mean per cent leaf damage of 64.38 and 50.22 respectively with a damage score of 5.

Table 22. Categorization of brinjal cultivars based on leaf damage caused by *Coccidohystrix insolita*

Cultivars	Leaf damage (%)	Damage score	Rating index
Haritha	50.22	5	Highly susceptible
Neelima	28.94	3	Moderately susceptible
Ponni	17.05	2	Moderately resistant
Pink Long	34.80	4	Susceptible
Udit	64.38	5	Highly susceptible
Green Long	32.49	4	Susceptible
Pusa Purple Long	8.96	1	Resistant
Pusa Kaushal	25.16	3	Moderately susceptible
Pusa Uttam	13.25	2	Moderately resistant
Pusa Shyamla	28.65	3	Moderately susceptible

4.4.1.4. *Estimation of Natural Enemy Population*

The population of natural enemies in different cultivars of brinjal were recorded at 65, 75, 85 and 95 days after transplanting and are depicted in Table 23.

The population of natural enemies in different brinjal cultivars showed no significant differences at 65 days after transplanting whereas the mean population of natural enemies at 75 days after transplanting exhibited significant differences. The mean population of natural enemies varied from 0.00 to 4.00 at 75 days after transplanting. The highest population of natural enemies were recorded in the cultivar, Haritha (4.00 plant^{-1}) which was statistically on par with Pusa Shymala (2.33 plant^{-1}), Udit (2.00 plant^{-1}) and Pusa Kaushal (2.00 plant^{-1}). The cultivar Green Long exhibited the lowest population of natural enemies (0.00 plant^{-1}) which was statistically on par with Ponni (0.67 plant^{-1}), Pink Long (1.33 plant^{-1}) and Pusa Uttam (1.33 plant^{-1}).

The mean population of natural enemies per plant in different brinjal cultivars at 85 days after transplanting ranged from 0.33 to 4.67. The cultivar Haritha attracted the highest number of natural enemies (4.67 plant^{-1}) which was statistically on par with the hybrid cultivar, Udit (3.67 plant^{-1}). It was followed by Neelima (2.33 plant^{-1}) which was statistically on par with the Pink Long (2.00 plant^{-1}), Pusa Purple Long (2.00 plant^{-1}) and Pusa Shyamla (2.00 plant^{-1}). The lowest mean population of natural enemies were recorded in Pusa Kaushal (0.33 plant^{-1}) which was statistically on par with Ponni (1.33plant^{-1}) and Pusa Uttam (1.33 plant^{-1}).

The brinjal cultivars differed significantly in the mean population of natural enemies at 95 days after transplanting and it ranged from 0.33 to 6.33. The highest mean population of natural enemies were recorded in Haritha (6.33 plant^{-1}) which was statistically on par with Pink Long (3.67 plant^{-1}). It was followed by the cultivars, Pusa Kaushal, Pusa Shymala, Udit and Green Long with mean population of natural enemies 3.00, 2.67, 2.33 and 2.00 plant^{-1} respectively which

Table 23. Population of natural enemies of *Coccidohystrix insolita* in different cultivars of brinjal

Cultivars	Natural enemies (No. plant^{-1})			
	65 DAT	75 DAT	85 DAT	95 DAT
Haritha	0.67 (1.05)	4.00 (2.11)^{a}	4.67 (2.28)^{a}	6.33 (2.60)^{a}
Neelima	1.00 (1.18)	1.67 (1.44)^{bc}	2.33 (1.65)^{bc}	1.67 (1.35)^{cd}
Ponni	0.67 (1.18)	0.67 (1.05)^{cd}	1.33 (1.27)^{cd}	0.33 (0.88)^{d}
Pink Long	0.00 (0.70)	1.33 (1.27)^{bcd}	2.00 (1.56)^{bc}	3.67 (2.03)^{ab}
Udit	0.33 (0.88)	2.00 (1.56)^{abc}	3.67 (2.04)^{ab}	2.33 (1.64)^{bc}
Green Long	0.00 (0.70)	0.00 (0.70)^{d}	1.67 (1.46)^{c}	2.00 (1.56)^{bc}
Pusa Purple Long	1.33 (1.27)	1.67 (1.46)^{bc}	2.00 (1.56)^{bc}	0.33 (0.88)^{d}
Pusa Kaushal	1.67 (1.46)	2.00 (1.53)^{abc}	0.33 (0.88)^{d}	3.00 (1.86)^{bc}
Pusa Uttam	0.67 (1.05)	1.33 (1.27)^{bcd}	1.33 (1.35)^{cd}	0.33 (0.88)^{d}
Pusa Shyamla	0.00 (0.70)	2.33 (1.68)^{ab}	2.00 (1.56)^{bc}	2.67 (1.76)^{bc}
SE m ±	-	0.12	0.12	0.18
CD(0.05)	NS	0.594	0.550	0.632

*DAT –Days After Transplanting

**Figures in parentheses are √x+0.5 transformed values

*** Means followed by the same letter(s) not significantly different

were statistically on par. The lowest population of natural enemies were observed in the cultivar Pusa Purple Long (0.33 plant^{-1}) which was statistically on par with Ponni (0.33 plant^{-1}), Pusa Uttam (0.33 plant^{-1}) and Neelima (1.67 plant^{-1}).

The mean population of spiders in different brinjal cultivars at 65, 75, 85 and 95 days after transplanting were recorded and the data is given in Table 24.

The brinjal cultivars exhibited no significant differences in the mean population of spiders at 65 and 75 days after transplanting whereas it differed significantly among the cultivars at 85 days after transplanting. The mean population of spiders ranged from 0.00 to 3.00. The cultivar Haritha occupied the highest mean population of spiders (3.00 plant^{-1}) which was statistically on par with Pusa Purple Long (2.67plant^{-1}), Ponni (1.67plant^{-1}), Pink Long (1.33 plant^{-1}), Neelima (1.33 plant^{-1}), Green Long (1.33 plant^{-1}) and Udit (1.33 plant^{-1}). The lowest population of spiders were observed in the cultivar, Pusa Kaushal (0.00) which was statistically on par with Pusa Uttam (1.00 plant^{-1}) and Pusa Shymala (1.00 plant^{-1}).

The tested brinjal cultivars showed significant differences in the mean population of spider at 95 days after transplanting and it varied from 0.70 to 1.58. The highest population of spider was recorded in the cultivar Green Long which was statistically on par with Ponni, Haritha, Pink Long, Pusa Shymala, Pusa Purple Long and Neelima with a mean population of spiders 2.00, 2.00, 1.33, 1.33, 1.00, 1.00 and 1.00 plant^{-1} respectively. The cultivar, Pusa Kaushal recorded the lowest population of spiders (0.00) which was statistically on par with the cultivar, Udit (0.33 plant^{-1}), Pusa Uttam (0.33 plant^{-1}), Neelima (1.00 plant^{-1}) and Pusa Purple Long (1.00 plant^{-1}).

Table 24. Population of spiders in different cultivars of brinjal

Cultivars	Spider (No. plant^{-1})			
	65 DAT	75 DAT	85 DAT	95 DAT
Haritha	0.00 (0.70)	0.33 (0.88)	3.00 (1.86)^{a}	1.33 (1.35)^{ab}
Neelima	0.33 (0.88)	0.00 (0.70)	1.33 (1.35)^{ab}	1.00 (1.18)^{abc}
Ponni	0.33 (0.88)	1.00 (1.18)	1.67 (1.46)^{ab}	2.00 (1.56)^{a}
Pink Long	0.67 (0.99)	1.33 (1.35)	1.33 (1.35)^{ab}	1.33 (1.35)^{ab}
Udit	0.67 (1.05)	0.33 (0.88)	1.33 (1.27)^{abc}	0.33 (0.88)^{bc}
Green Long	0.33 (0.88)	0.67 (1.05)	1.33 (1.29)^{abc}	2.00 (1.58)^{a}
Pusa Purple Long	0.67 (0.99)	1.33 (1.29)	2.67 (1.77)^{ab}	1.00 (1.18)^{abc}
Pusa Kaushal	0.67 (0.99)	0.00 (0.70)	0.00 (0.70)^{c}	0.00 (0.70)^{c}
Pusa Uttam	1.00 (1.18)	0.33 (0.88)	1.00 (1.18)^{bc}	0.33 (0.88)^{bc}
Pusa Shyamla	1.33 (1.35)	0.67 (0.99)	1.00 (1.18)^{bc}	1.00 (1.23)^{ab}
SE m ±	-	-	0.10	0.09
CD (0.05)	NS	NS	0.608	0.493

*DAT –Days After Transplanting

**Figures in parentheses are √x+0.5 transformed values

*** Means followed by the same letter(s) not significantly different

4.4.2. Biophysical Parameters Mediating Tri-trophic Interaction in Brinjal

The biophysical traits of plants played a significant role in maintaining the tri-trophic relations in an ecosystem by meddling in the activity of pests and their natural enemies. The biophysical characters of different brinjal cultivars is depicted in Table 25.

4.4.2.1 *Trichome Density*

Among the tested cultivars, the trichome density on the leaf varied from 416.67 to 1100.00 /cm^2 of the leaf surface. The highest trichome density was recorded with the cultivar, Udit (1100.00 /cm^2) which was significantly different from all other cultivars. The second highest trichome density was observed in the cultivar Haritha (900.00 /cm^2) which was statistically different from other cultivars. It was followed by Pink long, Green Long, Pusa Uttam and Ponni with trichome densities 783.33, 775.00, 758.33 and 733.33 /cm^2 respectively. The lowest trichome density was noted in the cultivar Pusa Kaushal (416.67 /cm^2) which was statistically on par with Pusa Purple Long (500.00 /cm^2). It was followed by the cultivar Neelima and Pusa Shymala with trichome densities 658.33 and 666.67 /cm^2 respectively.

4.4.2.2 *Leaf Thickness*

The leaf thickness measured from the brinjal cultivars ranged from 0.17 to 0.29 mm. Among the tested cultivars, Ponni recorded the highest leaf thickness of 0.29 mm which was statistically on par with Neelima (0.28 mm) and Pusa Uttam (0.27 mm). It was followed by Pusa Purple Long (0.23 mm) which was statistically on par with Pusa Shymala, Haritha, Green Long and Pink Long (leaf thickness of 0.22, 0.20, 0.19 and 0.19 mm respectively). The lowest leaf thickness was noted in the cultivar Pusa Kaushal (0.17 mm) which was statistically on par with Udit (0.17mm).

4.4.2.3 *Length Width Ratio of Leaf*

The length width ratio of the leaf ranged from 1.32 to 1.97 among the brinjal cultivars. The highest length width ratio of leaf was observed in the cultivar Udit (1.97) which was statistically on par with Pusa Shymala (1.94). It was followed by Pink Long (1.69) which was statistically on par with Ponni (1.67) and Haritha (1.60). The lowest length width ratio was observed in the cultivar Pusa Uttam (1.32) which was statistically on par with Neelima, Pusa Kaushal, Pusa Purple Long and Green Long (1.41, 1.41, 1.42 and 1.46 respectively).

4.4.2.4 *Number of Branches*

The number of branches in the plant varied from 2.33 to 13.33 among the tested brinjal cultivars. The cultivar Pink Long showed the highest number of branches (13.33 plant^{-1}) which was statistically on par with the cultivar Pusa Shymala (13.33 plant^{-1}) and Neelima (11.00 plant^{-1}). It was followed by Ponni, Green Long and Udit (10.33, 10.33 and 8.67 branches plant^{-1} respectively). The cultivar Pusa Uttam with the lowest number of branches plant^{-1} (2.33) which was statistically on par with Pusa Purple Long (4.00 plant^{-1}).

4.4.2.5 *Plant Height*

Among the tested cultivars**,** plant height ranged from 62.67 to 110.33 cm. The cultivar Neelima (110.33 cm) recorded the highest plant height which was statistically on par with Green Long (109.33 cm) and Ponni (106.00 cm). It was followed by Pusa Purple Long which was statistically on par with Pusa Kaushal with a plant height of 94.00 and 92.67 cm respectively. The cultivar**,** Udit showed lowest plant height of 62.67 cm which was statistically on par with Haritha (65.33 cm). It was followed by Pusa Shymala, Pusa Uttam and Pink Long (78.00, 86.00 and 87.67 cm respectively).

Table 25. Biophysical parameters of brinjal cultivars infested by *Coccidohystrix insolita.*

Cultivar	Trichome density number/ cm^2 of leaf surface	Leaf thickness (mm)	Length width ratio of leaf	Number of branches $plant^{-1}$	Plant height (cm)
Haritha	900.00^{b}	0.20^{bc}	1.60^{bc}	6.67^{de}	65.33^{ef}
Neelima	658.33^{e}	0.28^{a}	1.41^{d}	11.00^{ab}	110.33^{a}
Ponni	733.33^{c}	0.29^{a}	1.67^{b}	10.33^{bc}	106.00^{ab}
Pink Long	783.33^{cde}	0.19^{bc}	1.69^{b}	13.33^{a}	87.67^{cd}
Udit	1100.00^{a}	0.17^{c}	1.97^{a}	8.67^{bcd}	62.67^{f}
Green Long	775.00^{cd}	0.19^{bc}	1.46^{cd}	10.33^{bc}	109.33^{a}
Pusa Purple Long	500.00^{f}	0.23^{b}	1.42^{d}	4.00^{ef}	94.00^{bc}
Pusa Kaushal	416.67^{f}	0.17^{c}	1.41^{d}	8.00^{cd}	92.67^{bc}
Pusa Uttam	758.33^{cde}	0.27^{a}	1.32^{d}	2.33^{f}	86.00^{cd}
Pusa Shyamala	666.67^{de}	0.22^{b}	1.94^{a}	13.33^{a}	78.00^{de}
CD(0.05)	116.611	0.039	0.170	2.919	14.054

* Means followed by the same letter(s) not significantly different

On comparing the biophysical parameters, the highly susceptible cultivar Udit was recorded with the highest trichome density, the highest length width ratio, the lowest plant height and the lowest leaf thickness whereas the cultivar Pusa Uttam was noted with the lowest length width ratio and length of plants.

4.4.2.6 *Biophysical Factors of Brinjal Cultivars with Mean Population of Mealybug and Natural Enemies*

The correlation between biophysical parameters of brinjal cultivars with the mean population of mealybugs and their natural enemies were evaluated and regression equations were worked out and are depicted in Table 26.

Among the various biophysical parameters, trichome density, length width ratio of leaf and number of branches plant^{-1} exhibited a significant positive correlation with the mean population of mealybugs with correlation coefficients of 0.692, 0.702 and 0.644 respectively. The leaf thickness and plant height showed a non-significant negative correlation with mean population of mealybugs with correlation coefficients -0.447 and -0.456. The regression equation obtained was $Y = 0.099\ X_1 - 124.602\ X_2 - 57.811\ X_3 + 7.784\ X_4 - 0.856\ X_5 + 140.088$.

On analyzing the correlation between biophysical parameters of brinjal cultivars and the mean population of natural enemies, plant height exhibited a significant negative correlation with a correlation coefficient of -0.691 whereas leaf thickness and number of branches plant^{-1} revealed a non- significant negative correlation with correlation coefficients -0.419 and -0.009 respectively. The trichome density and length width ratio of leaf showed a positive non-significant correlation with correlation coefficients 0.339 and 0.259. The regression equation obtained was $Y = 1.430\ X_2 - 4.099\ X_3 + 0.202\ X_4 - 0.076\ X_5 + 12.687$.

4.4.3 Biochemical Parameters Mediating Tri-trophic Interaction in Brinjal

The biochemical factors of host plants exerted a significant impact on the tri-trophic interactions. The data on biochemical parameters of different brinjal cultivars is depicted in Table 27.

Table 26. Correlation of biophysical parameters of brinjal cultivars with mean population of mealybug and natural enemies

Sl. No.	Correlation between independent and dependent variables	Correlation coefficient	Coefficient of determination (R^2)	Regression equation
1	Trichome density v/s mean population of mealy bugs	0.692*	0.933	$Y = 0.099\ X_1 - 124.602\ X_2 - 57.811\ X_3 + 7.784\ X_4 - 0.856\ X_5 + 140.088$
2	Leaf thickness v/s mean population of mealy bugs	-0.447		
3	Length width ratio of leaf v/s mean population of mealy bugs	0.702*		
4	Number of branches plant^{-1} v/s mean population of mealy bugs	0.644*		
5	Plant height v/s mean population of mealy bugs	-0.456		
6	Trichome density v/s mean population of coccinellids	0.339	0.699	$Y= 1.430\ X_2 - 4.099\ X_3 + 0.202\ X_4 - 0.076\ X_5 +12.687$
7	Leaf thickness v/s mean population of coccinellids	-0.419		
8	Length width ratio of leaf v/s mean population of coccinellids	0.259		
9	Number of branches plant^{-1} v/s mean population of coccinellids	-0.009		
10	Plant height v/s mean population of coccinellids	-0.691*		

*Significant at 5% level

4.4.3.1 *Total Phenol Content*

The total phenol content varied from 1.14 to 4.62 mg g^{-1} among the tested brinjal cultivars. The cultivar Pusa Uttam recorded the highest phenol content (4.62 mg $g^{-1)}$ which was statistically superior to all other cultivars. The second highest phenol concentration was noted in the cultivar Neelima (3.99 mg g^{-1}) which was statistically on par with Green Long (3.90 mg g^{-1})) and Pusa Purple Long (3.49 mg g^{-1}). It was followed by Pusa Kaushal (3.36 mg g^{-1}) which was statistically on par with Ponni (3.21 mg g^{-1}) and Pusa Shymala (3.11 mg g^{-1}). The lowest concentration of phenol was recorded in the cultivar Udit (1.14 mg g^{-1}) which was statistically on par with Pink Long (1.49 mg g^{-1}).

4.4.3.2 *Total Protein Content*

The total protein content ranged from 6.36 to 12.06 mg g^{-1} among the tested brinjal cultivars. The highest protein content was recorded with Pusa Kaushal (12.06 mg g^{-1}) which was statistically on par with Neelima (10.43 mg g^{-1}). It was followed by Pusa Purple Long (9.86 mg g^{-1}) which was statistically on par with Pusa Uttam, Haritha, Green Long and Ponni (9.61, 9.43, and 8.96 mg g^{-1} respectively). The lowest protein content was recorded with the cultivar Pink Long (6.36 mg g^{-1}) which was significantly different from all other cultivars. It was followed by Pusa Shymala (8.23 mg g^{-1}) and Udit (8.29 mg g^{-1}).

4.4.3.3 *Total Reducing Sugar*

The concentration of reducing sugar varied from 1.19 to 2.87 mg g^{-1} among the tested cultivars. The cultivar, Pusa Purple Long showed the highest reducing sugar (2.87 mg g^{-1}) which was statistically on par with Haritha (2.76 mg g^{-1}), Pusa Shymala (2.53 mg g^{-1}), Green Long (2.52 mg g^{-1}), Ponni (2.46 mg g^{-1}), Udit (2.45 mg g^{-1}), Pusa Kaushal (2.30 mg g^{-1}) and Pink Long (2.14 mg g^{-1}). The concentration of reducing sugars was found to be the lowest in Pusa Uttam (1.19 mg g^{-1}) which was statistically on par with Neelima (1.69 mg g^{-1}).

Table 27. Biochemical parameters of brinjal cultivars infested by *Coccidohystrix insolita.*

Cultivars	Total phenol (mg g^{-1})	Total protein (mg g^{-1})	Reducing sugar (mg g^{-1})	Total chlorophyll (mg g^{-1})	Total carotenoids (mg g^{-1})
Haritha	2.00^{e}	9.43^{bc}	2.76^{a}	2.31^{b}	0.60^{cd}
Neelima	3.99^{b}	10.43^{ab}	1.69^{bc}	1.89^{cd}	0.72^{bcd}
Ponni	3.21^{d}	8.93^{bc}	2.46^{ab}	1.59^{e}	0.60^{cd}
Pink Long	1.49^{ef}	6.36^{d}	2.14^{ab}	2.05^{c}	0.81^{b}
Udit	1.14^{f}	8.29^{c}	2.45^{ab}	2.84^{a}	0.67^{bcd}
Green Long	3.90^{bc}	8.96^{bc}	2.52^{a}	1.56^{e}	0.59^{d}
Pusa Purple Long	3.49^{bcd}	9.86^{bc}	2.87^{a}	2.09^{c}	0.80^{b}
Pusa Kaushal	3.36^{cd}	12.06^{a}	2.30^{ab}	1.68^{de}	0.61^{cd}
Pusa Uttam	4.62^{a}	9.61^{bc}	1.19^{c}	1.55^{e}	0.96^{a}
Pusa Shymala	3.11^{d}	8.23^{c}	2.53^{a}	2.07^{c}	0.73^{bc}
CD(0.05)	0.597	1.888	0.804	0.221	0.14

* Means followed by the same letter (s) not significantly different

4.4.3.4 *Total Chlorophyll Content*

The total chlorophyll content of leaves in different cultivars ranged from 1.55 to 2.84 mg g^{-1}. The chlorophyll content was found to be highest in the cultivar Udit (2.84 mg g^{-1}) which was statistically superior to all other cultivars. The cultivar, Haritha showed the second highest chlorophyll content (2.31 mg g^{-1}) which was statistically different from other cultivars. It was followed by Pusa Purple Long, Pusa Shymala and Pink Long with chlorophyll content 2.09, 2.07 and 2.05 mg g^{-1} respectively. The lowest chlorophyll content was noted with the cultivar Pusa Uttam (1.55 mg g^{-1}) which was statistically on par with Green Long (1.56 mg g^{-1}), Ponni (1.59 mg g^{-1}) and Pusa Kaushal (1.68 mg g^{-1}).

4.4.3.5 *Total Carotenoid Content*

The carotenoid content of brinjal cultivars varied from 0.59 to 0.96 mg g^{-1}. The highest carotenoid content was noted with the cultivar Pusa Uttam (0.96 mg g^{-1}) which was statistically superior from all other cultivars. It was followed by Pink Long which was statistically on par with Pusa Purple Long, Pusa Shymala, Neelima and Udit with carotenoid content of 0.81, 0.80, 0.73, 0.72 and 0.67 mg g^{-1} respectively. The lowest carotenoid content was recorded with Green Long (0.59 mg g^{-1}) which was statistically on par with Ponni (0.60 mg g^{-1}), Haritha (0.60 mg g^{-1}) and Pusa Kaushal (0.61 mg g^{-1}).

On comparing the biochemical parameters, Udit was recorded with lowest phenol content and highest chlorophyll content whereas Pusa Uttam was recorded with the highest phenol contel, carotenoid content and lowest sugar content and protein content.

4.4.3.6 *Biochemical parameters of Brinjal Cultivars with Mean Population of Mealybug and Natural Enemies*

Correlation analysis of the biochemical parameters of brinjal cultivars with the mean population of mealybugs and natural enemies were carried out and regression equations were generated and the details are given in Table 28.

Table 28. Correlation of biochemical parameters with mean population of mealybug and natural enemies

Sl. No.	Correlation between independent and dependent variables	Correlation coefficient	R^2	Regression equation
1	Total phenol v/s mean population of mealy bugs	-0.731*	0.810	$Y= 2.636\ X_1 - 135.527\ X_2 - 21.739\ X_3 + 55.931\ X_4 - 235.137\ X_5 + 305.847$
2	Total protein v/s mean population of mealy bugs	-0.372		
3	Reducing sugar v/s mean population of mealy bugs	0.273		
4	Total chlorophyll v/s mean population of mealy bugs	0.647*		
5	Carotenoids v/s mean population of mealy bugs	-0.347		
6	Total phenol v/s mean population of coccinellids	-0.580	0.497	$Y= -0.308\ X_1 +1.744\ X_2 - 0.160\ X_3 + 0.892\ X_4 - 0.496\ X_5 + 0.013$
7	Total protein v/s mean population of coccinellids	0.116		
8	Reducing sugar v/s mean population of coccinellids	0.345		
9	Total chlorophyll v/s mean population of coccinellids	0.625		
10	Carotenoids v/s mean population of coccinellids	-0.287		

*Significant at 5% level

Among the biochemical parameters of brinjal cultivars, total phenol content exhibited a significant negative correlation with the mean population of mealybugs with a correlation coefficient of -0.731 whereas total chlorophyll content showed a significant positive correlation with a correlation coefficient of 0.647. The biochemical parameters such as total protein content and carotenoid content exhibited a non-significant negative correlation with the mean population of mealybugs with correlation coefficients -0.372 and -0.347 respectively whereas concentration of reducing sugars showed a non-significant positive correlation with a correlation coefficient of 0.273. The regression equation obtained was $Y= 2.636 X_1 -135.527 X_2 - 21.739 X_3 +55.931 X_4 - 235.137 X_5 + 305.847$.

The correlation between biochemical parameters of brinjal cultivars with the mean population of natural enemies revealed that total phenol and carotenoid content exhibited a non-significant negative correlation with correlation coefficients -0.580 and -0.287 respectively whereas total protein content, reducing sugar and total chlorophyll content showed a non-significant positive correlation with correlation coefficients 0.116, 0.345, 0.625 respectively. The regression equation developed was $Y= - 0.308 X_1 + 1.744 X_2 -0.160 X_3 + 0.892 X_4 - 0.496 X_5 + 0.013$.

4.4.4 Info- Chemical Mediated Tri-trophic Interaction in Brinjal, Mealybug and Natural Enemies

The info-chemical mediated tri-trophic relationships among brinjal cultivars, mealybug and its natural enemies were studied by using multi-armed olfactometer and Y- shaped olfactometer assay. The synomonal compound elicited by the brinjal cultivars and kairomonal compounds emanated from mealybugs were identified using GC-MS.

4.4.4.1 *Response of C. zastrowi sillemi to Synomonal Compounds of Brinjal*

The synomonal compounds of mealybug infested brinjal cultivars were extracted and the response of the natural enemy, *C. zastrowi sillemi* was evaluated.

4.4.4.1.1 *Multi-armed Olfactometer Assay*

The orientation response of *C. zastrowi sillemi* adults towards the synomonal compounds of ten brinjal cultivars using a multi-armed olfactometer was evaluated and the results are presented in Table 29.

The results revealed that the cultivar Udit attracted the highest number of natural enemies (2.87) which was statistically superior to all other treatments. The second highest number of *C. zastrowi sillemi* were oriented towards the arm containing the synomonal compounds of the cultivar Haritha (2.26) which was statistically different from other cultivars. This was followed by the cultivar, Ponni (0.83) which was statistically on par with Pink Long (0.67). However, significantly lowest number of *C. zastrowi sillemi* were oriented towards the cultivar, Pusa Uttam (0.28) which was statistically on par with n-hexane, Pusa Shymala, Pusa Purple Long, Neelima and Green Long (0.38, 0.42, 0.45, 0.47 and 0.47 respectively).

4.4.4.1.2 Y- tube Olfactometer Assay

The relative response of *C. zastrowi sillemi* adults to the synomonal extracts of healthy and mealybug infested brinjal cultivar Udit (most preferred cultivar) was evaluated in a Y- tube olfactometer and the results are presented in Table 30.

The results revealed that *C. zastrowi sillemi* adults showed more preference towards the synomonal compounds of mealybug infested plants (5.20) than that of healthy synomonal extracts (2.90).

4.4.4.2 Response of C. zastrowi sillemi to Kairomonal Compounds of Mealybug C. insolita.

The response of *C. zastrowi sillemi* adults towards the kairomonal compounds of mealybug, *C. insolita* was evaluated in a Y- tube olfactometer and the findings are presented in Table 31.

Table 29. Response of *Chrysoperla zastrowi sillemi* to synomonal compounds of brinjal cultivars

Treatments	Number of *C. zastrowii* attracted
Haritha	2.26
Neelima	0.47
Ponni	0.83
Pink Long	0.67
Udit	2.87
Green Long	0.47
Pusa Purple Long	0.45
Pusa Kaushal	0.53
Pusa Uttam	0.28
Pusa Shyamla	0.42
n-hexane	0.38
CD (0.05)	0.239

Table 30. Response of *Chrysoperla zastrowi sillemi* towards synomonal compounds of healthy and mealy bug infested brinjal cultivar, Udit.

Treatments	Synomones from healthy plants	Synomones from Mealybug infested plants
Mean	2.90	5.20
Standard Deviation	0.876	1.135
t test value (0.01)	3.25**	

Table 31. Response of the natural enemy *Chrysoperla zastrowi sillemi* towards kairomonal compounds of *Coccidohystrix insolita*

Treatments	Kairomones from mealybug, *C. insoilta*	n-hexane (control)
Mean	5.90	2.80
Standard Deviation	1.101	1.033
t test value (0.01)	3.25**	

The results revealed that the highest mean number of adult lacewings were attracted to the arm containing kairomonal compounds of mealybug (5.90) compared to the arm containing n-hexane (2.80).

The cultivar Udit (mealybug infested) was recorded with a total of 11 compounds, of which the compound diisooctyl phthalate was recorded with the highest area (33.25%) followed by hexadecane (16.56 %), heptadecane, 2,6,10,15-tetramethyl (11.97%), beta-Eudesmene (9.53%), dodecane (8.87%), eremophilene (5.19%), decane, 2,3,5,8-tetramethyl (4.60%), Octane, 3-ethyl-2,7-dimethyl (4.00%), N-(trifluoroacetyl)-N,O,O',O''-tetrakis (trimethylsilyl) norepinephrine (2.40%), nonane, 3,7-dimethyl (2.36 %) and oxalic acid, allyl decyl ester (1.27%) (Table 32).

The cultivar Pusa Uttam was recorded with a total of five compounds, of which diisooctyl phthalate was the most dominant compound (45.60%) followed by tetradecane (21.94 %) and hexadecane (15.97%). The other volatile compounds identified from Pusa Uttam in low concentration were dodecane (10.82%) and heptadecane (5.66%) (Table 33).

The healthy plant synomonal extracts of the cultivar Udit was recorded with a total of nine compounds, of which tetratetracontane was noted as the most dominant compound (43.90%) followed by hexatriacontane (20.79%) and 2-methyloctacosane (10.32%). The other volatile compounds recorded were tetradecane (8.63%), Hexadecane (7.23%), heptadecane (3.76%), dodecane (2.83%), thymine glycol (1.4%) and 13-hexyloxacyclotridecan-2-one (1.14%) (Table 34).

Table 32. Hydrocarbon profile of the cultivar Udit infested by *Coccidohystrix insolita*

Sl.No	Name of compound	Area (%)
1	Octane, 3-ethyl-2,7-dimethyl-	4.00
2	Dodecane	8.87
3	beta.-Eudesmene	9.53
4	Eremophilene	5.19
5	Oxalic acid, allyl decyl ester	1.27
6	Hexadecane	16.56
7	N-(Trifluoroacetyl)-N,O,O',O''-tetrakis (trimethylsilyl) norepinephrine	2.40
8	Nonane, 3,7-dimethyl	2.36
9	Heptadecane, 2,6,10,15-tetramethyl-	11.97
10	Decane, 2,3,5,8-tetramethyl-	4.60
11	Diisooctyl phthalate	33.25

Table 33. Hydrocarbon profile of cultivar Pusa Uttam infested by *Coccidohystrix insolita*

Sl. No	Name of compound	Area (%)
1	Dodecane	10.82
2	Tetradecane	21.94
3	Hexadecane	15.97
4	Heptadecane	5.66
5	Diisooctyl phthalate	45.60

Table 34. Hydrocarbon profile of cultivar Udit (Healthy)

Sl. No	Name of compound	Area (%)
1	Thymine glycol	1.40
2	Dodecane	2.83
3	Tetradecane	8.63
4	Hexadecane	7.23
5	Heptadecane	3.76
6	13-Hexyloxacyclotridecan-2-one	1.14
7	Tetratetracontane	43.90
8	2-methyloctacosane	10.32
9	Hexatriacontane	20.79

Table 35. Hydrocarbon profile of the kairomonal compounds extracted from *Coccidohystrix insolita*

Sl. No	Name of compound	Area (%)
1	Undecane, 4,7-dimethyl	5.18
2	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris(trimethylsiloxy) tetrasiloxane	44.69
3	Dodecane	7.70
4	Cyclooctasiloxane, hexadecamethyl	18.20
5	Sulfurous acid, hexyl octyl ester	5.50
6	2-Bromo dodecane	6.36
7	Squalene	12.37

4.4.4.3.2 *Identification of Kairomonal Compounds using Gas-Chromatography-Mass Spectrometry*

The kairomonal compounds of *C. insolita* was identified through GCMS analysis and seven volatile compounds were present in the kairomonal extract. Among the compounds, 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris (trimethylsiloxy) tetrasiloxane was recorded as the most abundant one (44.69%), which was followed by cyclooctasiloxane, hexadecamethyl (18.20%), and squalene (12.37 %). Other volatile compounds recorded were dodecane (7.70 %), 2-Bromo dodecane (6.36 %, Sulfurous acid, hexyl octyl ester (5.50 %) and Undecane, 4,7-dimethyl (5.18 %) (Table 35).

Discussion

5. DISCUSSION

A study on "Mealybugs of vegetable ecosystems and tritrophic interactions of brinjal mealybugs" was conducted at the College of Agriculture, Vellayani during the year 2017 - 2020. The results of the study are discussed in this chapter.

5.1 COLLECTION AND IDENTIFICATION OF MEALYBUGS IN SOLANACEOUS AND CUCURBITACEOUS VEGETABLES

5.1.1 Documentation of Mealybugs Infesting Solanaceous and Cucurbitaceous Vegetables in Kerala

A purposive sampling of mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala revealed a total of six mealybug species. Besides, four mealybug species were also recorded from other vegetable crops in Kerala. Mealybugs are considered as an economically important pest that cause tremendous damage all over the world and about 41 species of mealybugs were recorded as pests of vegetables (Mani and Shivaraju, 2016). Vidya and Bhaskar (2017) recorded three mealybug species from the vegetable ecosystem of Kerala whereas Sreeja *et al.* (2018) documented four mealybug species infesting polyhouse vegetables in Kerala.

5.1.2 Identification of Mealybug Species Infesting Solanaceous and Cucurbitaceous Crops through Morphological Characterization

The exact identification of target pests is one of the important prerequisites in tri-trophic studies as the interactions may vary with each species. The collected mealybug species were identified using the taxonomic keys provided by Williams (2004).

The genera *Coccidohystrix* was reported with a single species, *C. insolita* in Kerala. Two species of *Coccidohystrix viz., C. eleusines* and *C. insolita* were noted in Southern Asia and the latter caused severe economic damage in crop

plants. The species *C. insolita* can be differentiated from *C. eleusines* by the absence of dorsal oral collar tubular ducts (Williams, 2004).

The striped mealybug, *F. virgata,* a cosmopolitan mealybug species was recorded from the vegetable crops in Kerala. Williams (1996) provided information about eight species of *Ferrisia* of New World origin and later Williams (2004) described two species of *Ferrisia viz., F. virgata* and *F. malvastra* from Southern Asia. *F. virgata* can be distinguished from *F. malvastra* by the presence of eight multilocular disc pores on abdominal segment VI in double rows and the dorsal ducts with a rim larger than a multilocular disc pore and with the setae located within the border of the rim. Kaydan and Gullan (2012) conducted a taxonomic revision of the genus *Ferrisia* and reported that the genera comprised of 18 species in the world.

The invasive alien pest *P. marginatus* was recorded to be infesting the vegetable crops in Kerala during the present study. The genus *Paracoccus* comprised of more than 80 species and about 10 species were reported from southern Asia (Ben -Dov, 1994). Certain species belongs to the genus *Paracoccus* was difficult to separate from the Genus *Pseudococcus,* but the presence of anal lobe bars separated the genus *Paracoccus.* A unique character that distinguishes *P. marginatus* from all other New World species was the presence of oral rim tubular ducts restricted only to the marginal regions of the body. The absence of translucent pores on the hind tibia was also considered as an important character of *P. marginatus* (Miller and Miller, 2002).

The cotton mealybug *P. solenopsis,* an economically important mealybug species infesting many crops all over the world, was documented as the dominant mealybug species infesting the vegetable crops in Kerala. The genera *Phenacoccus* was one of the most specious rich groups comprised of more than 200 species globally. The genus *Phenacoccus* can be easily discerned from other genera by the presence of short lanceolate setae and denticles on the claw (Williams, 2004). *P. solenopsis* differs from all other *Phenacoccus* species in South Asia by lacking the quinquelocular pores except *P. solani* with which it

shares striking similarity and were differentiated by the distribution of multilocular disc pores from abdominal segments VI to VIII and the presence of large flaccid circulus. However, *P. solani* and *P. solenopsis* were considered as environmental variations of a single species (Hodgson *et al.,* 2008). Similar results were also pointed out by Thomas and Ramamurathy (2014).

Two species of *Planococcus viz., P. citri* and *P. lilacinus* were documented from Kerala. The genera *Planococcus* was recorded with 40 species globally and about 18 species were documented from southern Asia. *P. citri* is one of the most common mealybugs in Southern Asia and it is difficult to distinguish from *P. minor* due to the variation in numbers of oral collar tubular ducts in the ventral region. *P. lilacinus* differs from *P. citri* by lacking multilocular disc pores in margins and submargins of the abdominal segments (rarely one or two present) whereas the latter possess multilocular disc pores in a single row from abdominal segments IV to VII (Williams, 2004: Mukhopadhyay, 2006).

The Jack Beardsley Mealybug, *P. jackbeardsleyi*, an invasive pest was documented from the vegetable crops in Kerala. The genus *Pseudococcus* was often confused with the genus *Dysmicoccus*, but the former possess oral collar rim tubular ducts whereas it was completely absent in the latter. *P. jackbeardsleyi* can be differentiated from other *Pseudococcus* species present in Southern Asia by the presence of discoidal pores around the eyes (Williams, 2004).

The four mealybug species collected from other vegetable crops were also identified.

The genera *Crisicoccus* was reported with eleven species in Southern Asia, in which a single species, *C. hirsutus* was recorded from Kerala which is distinguished by the absence of cerarii (Williams, 2004).

The pink mealybug *M. hirsutus,* a very destructive pest in grapevine, was also documented from the vegetable ecosystem of Kerala. The genera *Maconellicoccus* is reported with three species *viz., M. hirsutus, Maconellicoccus multipori* (Takahashi) and *Maconellicoccus ramchensis* Williams in Southern

Asia. (Williams, 1996). *M. hirsutus* is very similar to *M. multipori* and can be differentiated by the absence of circulus and oral collar ducts on the dorsum. *M. ramchensis* differed from *M. hirsutus* by the absence of oral collar tubular ducts on dorsum, deeply notched wide circulus and larger sized oral rim tubular ducts (Williams, 2004).

A single species of *R. iceryoides* was recorded from the vegetable crops in Kerala. The genus *Rastrococcus* is a remarkable genus with no similarity with any other genera except *Lankacoccus*, but differs in the presence of truncate conical setae rather than lanceolate setae in cerarii. *R. iceryoides* can be easily identified by the presence of distorted large trilocular pores, two pairs of ostioles and the presence of long flagellate setae flanking the anal ring (Williams, 2004).

5.1.3 Distribution of Mealybug Species in Kerala

Among the mealybugs, the most distributed species infesting vegetables in Kerala is striped mealybug, *F. virgata* followed by *P. solenopsis*. The wide host range coupled with favorable environmental conditions may offer better survival of these pests in the field. Sreeja *et al.* (2018) also recorded that *F. virgata* and *P. solenopsis* were the predominant species infesting vegetable crops in greenhouses of Kerala.

5.1.4. Study of Morphological and Molecular Variations in Brinjal Mealybug, *C. insolita*

A study on the morphological variations exhibited by the mealybug populations collected from different parts of Kerala revealed that the populations exhibited a great deal of morphological variations. PCA and CDA result also confirmed the morphological variations shown by the different populations of *C. insolita.*

However, the taxonomical and molecular characterization studies showed that all the populations belong to a single species, *C. insolita*. The morphological variations exhibited by the different populations of mealybugs may be environmental induced or seasonal variations. The study on morphological

variations of different populations of the mealybugs *viz., Pseudococcus calceolariae* (Maskell) and *Pseudococcus similans* (Lidgett) revealed that these species represented the phenotypic extremes of a single species and the morphological variations aroused from the difference in the microclimate in which they developed (Charles *et al.,* 2000). Hodgson *et al.* (2008) pointed out that the morphological variations shown by the populations of *P. solenopsis* in the Asian region may be due to environmental variation. A study conducted to explore the intraspecific variations in the morphological features of Indian populations of *P. solenopsis* also corroborated the present findings (Thomas and Ramamurthy, 2014).

5.1.5 Documentation of Host Range of Mealybugs Infesting Solanaceous and Cucurbitaceous Vegetables in Kerala

The study on the host range revealed a total of 113 plants under 73 genera belongs to 31 families as host plants of mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala. About 14 plants were recorded as host plants of mealybugs for the first time in the world. Being an economically important pest, broadening the host range of the pest was a serious concern that will adversely affect the sustainability of the ecosystem.

The plant families *viz.*, Asteraceae, Fabaceae, Malvaceae and Euphorbiaceae were severely infested by the mealybugs in Kerala (Fig.13). Ben-Dov (2006) also reported that the members of the family Asteraceae host a diverse array of mealybugs in the world. A wide host range of mealybugs was observed with weeds (31%) which was almost similar with vegetables (30%) and followed by ornamentals (21%) in Kerala (Fig. 14). The occurrence of this pest in food crops and the wide range of weed hosts that serve as alternate hosts during the offseason need to be addressed in next to no time.

The mealybug, *C. insolita* was recorded with seven host plants in Kerala and the predominant plant families infested by the mealybug were Malvaceae and Solanaceae. Ben-Dov (2013) also reported that Solanaceae and Malvaceae were

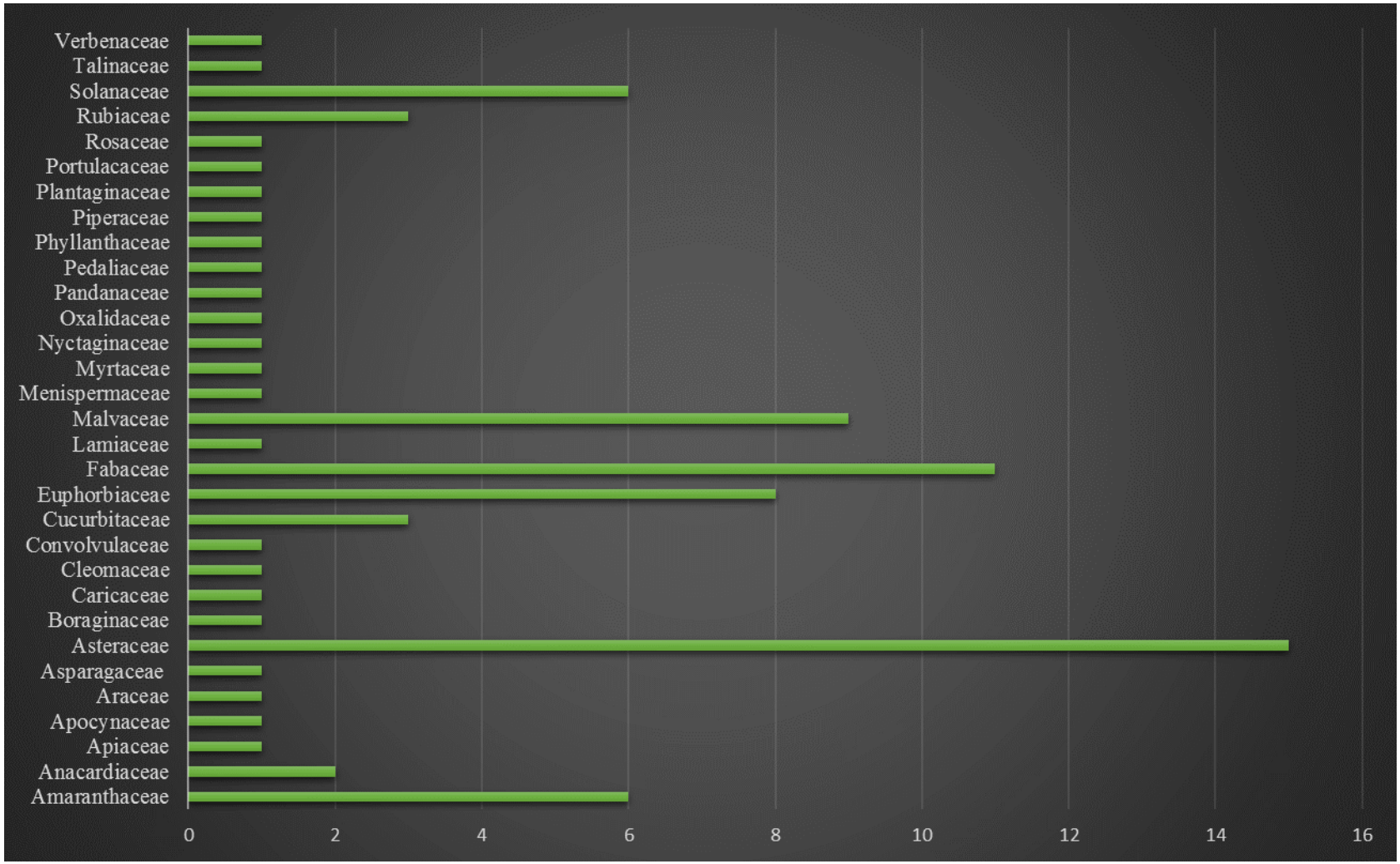


Fig. 13. Plant family wise host range of mealybugs infesting solanaceous and cucurbitaceous vegetables

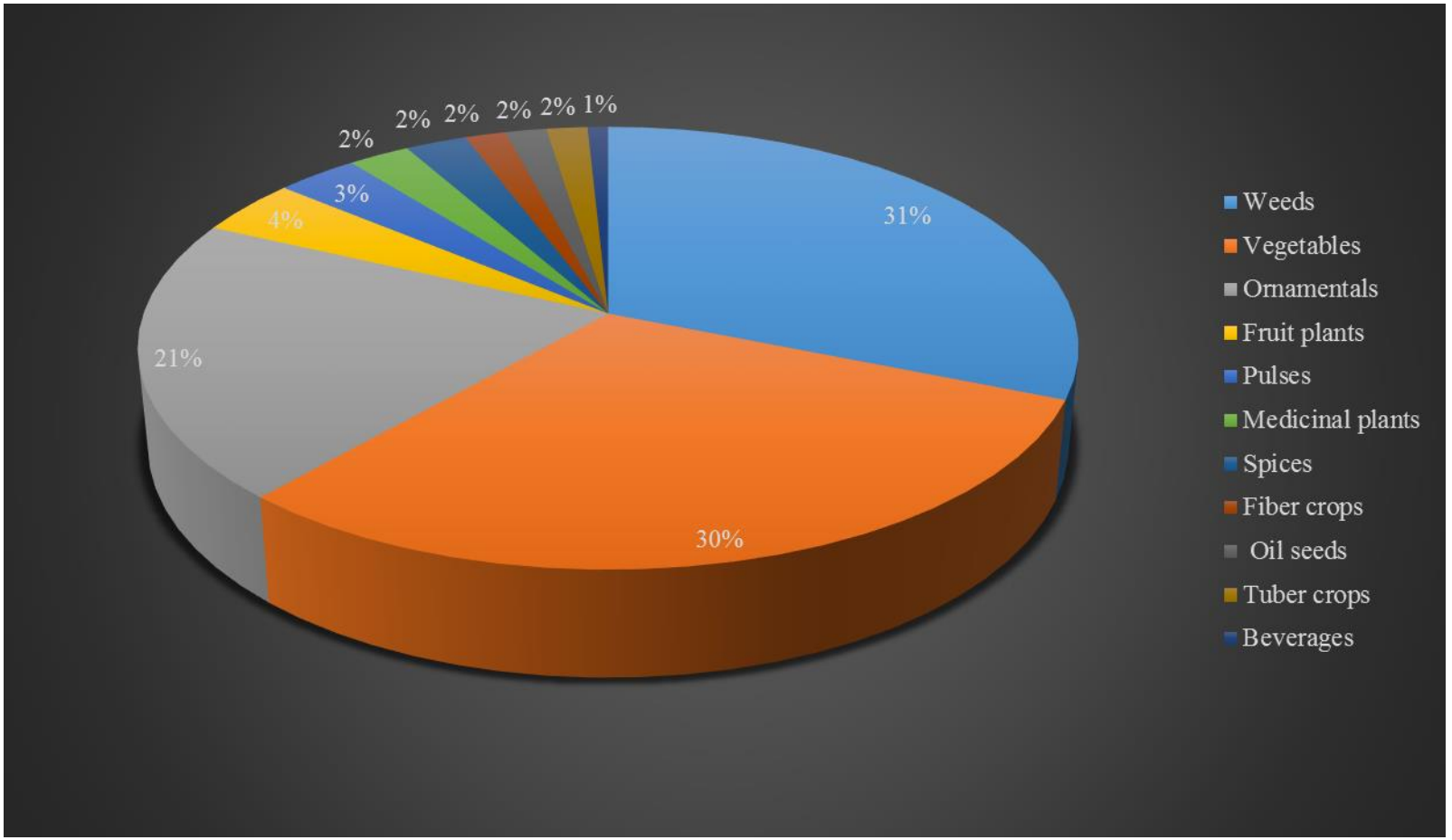


Fig. 14. Plant category wise host range of mealybugs infesting solanaceous and cucurbitaceous vegetables

mostly infested by the mealybug, *C. insolita* in the world. A study conducted by Jose (2017) recorded nine host plants of *C. insolita* from Kerala.

A total of 31 host plants were observed with the mealybug *F. virgata* and the plant families *viz.*, Fabaceae, Euphorbiaceae and Solanaceae were recorded with the maximum number of host plants in Kerala. *F. virgata* is a highly polyphagous pest with more than 200 host plants in the world, of which Fabaceae and Euphorbiaceae were the dominant groups damaged by the mealybug (Garcia-Morales *et al.*, 2016).

P. marginatus, an invasive alien pest was recorded with 11 host plants from Kerala. The majority of the host plants belongs to the families Malvaceae and Asteraceae. Similar observations were also made by Sakthivel *et al.* (2012) as they reported that the dominant families infested by the mealybug were Malvaceae, Solanaceae and Asteraceae in India. However, Chellappan *et al.* (2013b) recorded about 95 host plants from Kerala which comprised of Euphorbiaceae, Fabaceae and Asteraceae. The narrow host range of *P. marginatus* observed with this study may be due to the disappearance of this pest from the ecosystems of Kerala due to the intervention and successful establishment of biological control agents.

The cotton mealybug, *P. solenopsis*, was recorded as the dominant mealybug in Kerala with 42 host plants. The majority of the host plants belongs to the plant group Asteraceae followed by Solanaceae and Malvaceae. Similar observations were also made by Arif *et al.* (2009) and Vennila *et al.* (2010). A study conducted by Padmanabhan (2017) reported about 40 host plants in Kerala and the majority belongs to Asteraceae, Malvaceae and Solanaceae.

The mealybug *P. citri* was recorded with 13 host plants in Kerala. The Jackbeardsleyi mealybug, *P. jackbeardsleyi* was observed with nine host plants and the majority belongs to Euphorbiaceae and Solanaceae in Kerala. Being a recently reported invasive alien pest, a narrow host range was reported for *P. jackbeardsleyi* in Kerala.

The host shifting nature of mealybugs to annual herbaceous crops made the situation worse while considering the high reproduction rate and damaging potential of the pest.

5.1.6 Documentation of Natural Enemies of Mealybugs infesting Solanaceous and Cucurbitaceous Vegetables

Natural enemies play an important role in the regulation of the mealybug population in ecosystems. The understanding of the natural enemy fauna associated with the mealybug provides an insight into the various trophic level interactions in the ecosystem and also aids in developing better pest management options.

5.1.6.1 Predators

The important predators of mealybugs identified during the present study belong to the orders Coleoptera, Lepidoptera, Diptera and Neuroptera. These findings are in consonance with Fand and Suroshe (2015) and Shylesha and Mani (2016). A total of 20 species of predators were found associated with different mealybugs in Kerala, of which the predominant family was noted as Coccinellidae with 16 species. The genus *Scymnus* was recorded as the most specious group with seven species actively predating on different mealybugs in Kerala. Likewise, Vidya and Bhaskar (2017) reported that *Scymnus (Pullus) coccivora* was the predominant mealybug predator in Kerala. Manjushree *et al.* (2019) also documented *Scymnus* sp. as the dominant predator of *Dysmiococcus brevipes* in Kerala.

The lycaenid, *S. epius* was noted as a predator of five different mealybugs *viz., C. insolita, P. lilacinus, F. virgata, P. solenopsis* and *P. marginatus* in Kerala. Dinesh and Venkatesha (2011) documented *S. epius* as a dominant predator of *P. lilacinus* and *F. virgata* whereas Mani *et al.* (2012) reported *S. epius* as a voracious predator of *P. marginatus* in Kerala. Arve *et al.* (2013) recorded that the mealybug, *P. solenopsis* was actively predated by *S. epius*

whereas Arya (2015) recorded *S. epius* as a potential predator of *C. insolita* in Kerala.

A cecidomyiid species, *Diadiplosis* sp. was recorded as a predator of the mealybug *P. jackbeardsleyi* in Kerala. The genus *Diadiplosis*, a cosmopolitan genus, commonly distributed in the tropical regions were reported as a predator of several mealybugs and the species *Diadiplosis martinsensis* Culik and Ventura was recorded from *P. jackbeardsleyi* on pineapple and coffee (Culik and Ventura, 2013; Urso-Guimaraes *et al.,* 2020).

The larval stages of the genus *Cacoxenus* belong to the family Drosophilidae was observed on a new mealybug host, *P. jackbeardsleyi* in Kerala. Mani and Shivaraju (2016) reported that drosophilid predators play a supplementary role in the regulation of mealybug population. The genus *Cacoxenus* was previously reported in Kerala as a predator of *D. brevipes* on pineapple (Manjushree *et al.,* 2019).

The chrysopid predators are voracious feeders of mealybugs and a single species of *Chrysoperla* was recorded as a predator of the mealybug, *F. virgata* and *C. insolita* in Kerala. Adly *et al*. (2016) reported *Chrysoperla carnea* (Steph.) as a predator of *F. virgata* infesting Guava in Egypt. The *C. zastrowii sillemi* recorded a predatory efficiency of 66.87 *F. virgata* nymphs in its entire larval period that highlighted the importance of chrysopids in regulating the mealybug population in an ecosystem (Elango *et al.,* 2020).

Among the different mealybug species recorded from Kerala, the highest number of predators were observed in *C. insolita* (13 species) followed by *F. virgata* (5 species) (Fig.15). The major predators on *C. insolita* belongs to the family Coccinellidae with 12 species. The relatively high preference exhibited by coccinellids on *C. insolita* may be due to the predator-prey size relationship as most of the coccinellids were very small in size especially the genus *Scymnus* which was a dominant predator on mealybug *C. insolita*. The morphological peculiarities of the mealybug *viz.,* small size and less mobility favored the easy

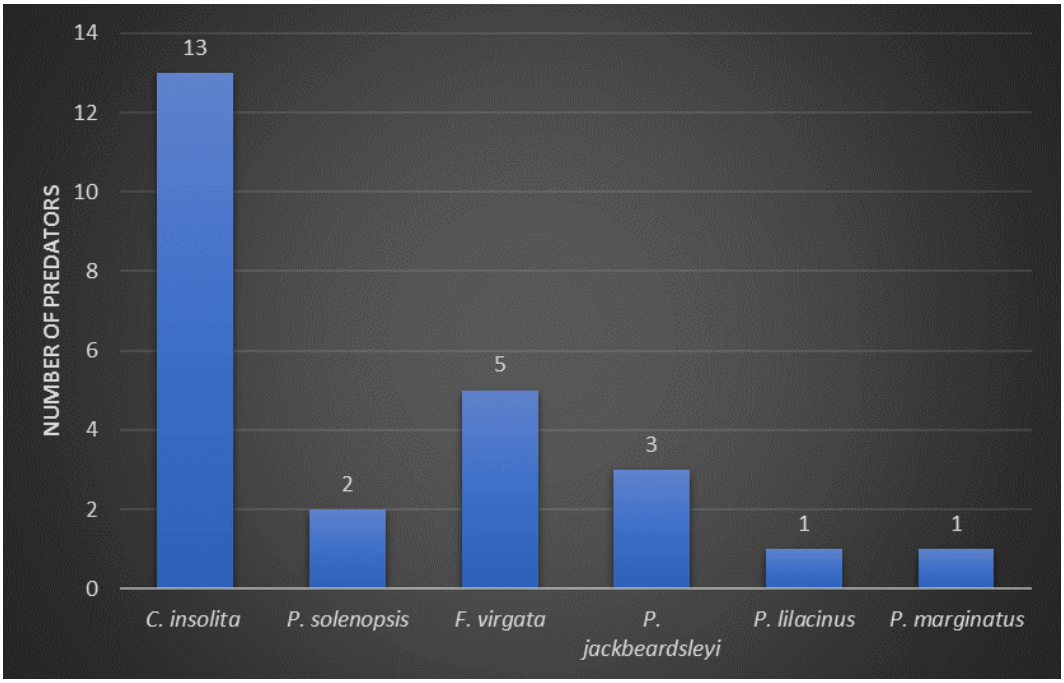


Fig. 15. Number of predators recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

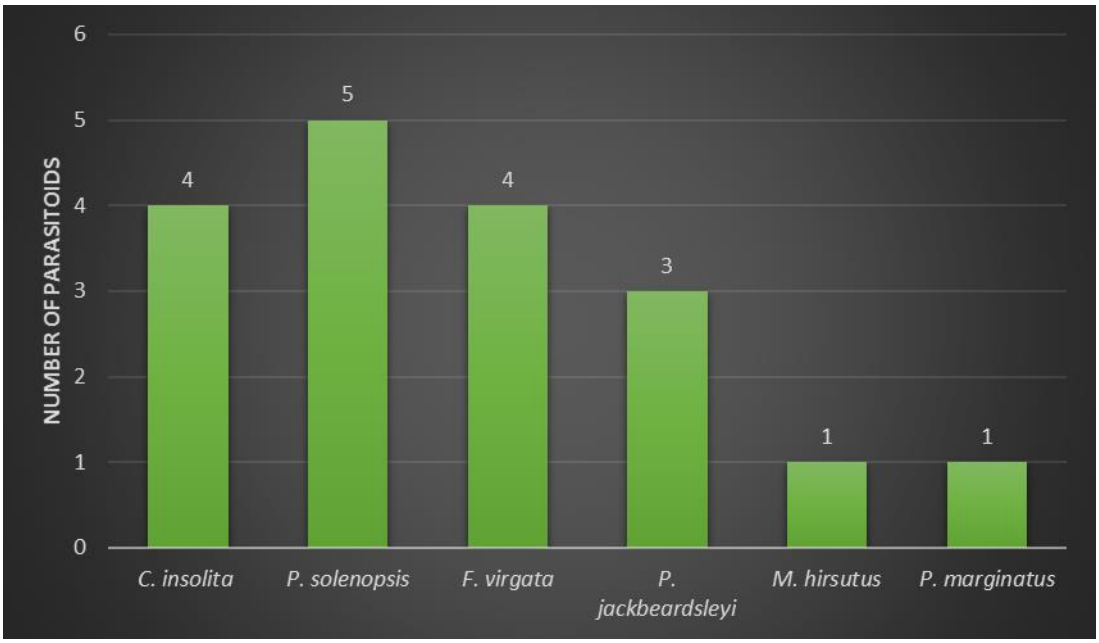


Fig 16. Number of parasitoids recorded from mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala

predation on *C. insolita.* Furthermore, most of the coccinellid grubs were observed to be feeding on the eggs in the long ovisac of *C. insolita* that provided protection from harsh external factors. Kitherian *et al.* (2018) also pointed out that predator-prey size ratio and morphological adaptations aids in a higher predation rate of reduviid predators on *P. solenopsis.*

5.1.6.2 Parasitoids

Mealybugs were parasitized by an array of hymenopteran parasitoids and about 11 species belong to five families were recorded from Kerala. The majority of mealybug parasitoids reported belongs to the family Encyrtidae followed by Eriaporidae in Kerala. Similar observations were also made by Hayat (2006) and Mani and Shivaraju (2016).

The family Encyrtidae was noted with five parasitoids of mealybugs in Kerala whereas Hayat (2006) recorded a total of 62 encyrtid parasitoid species from India. Among the encyrtids, the most common genera was *Aenasius* comprised of two species *viz., A. arizonensis* parasitizing on *P. solenopsis* and *F. virgata* and *A. advena* parasitizing on *F. virgata. A. arizonensis,* an indigenous parasitoid, was recorded as the most successful hymenopteran biocontrol agent of mealybug which recorded 90 per cent parasitism in *P. solenopsis* (Tanwar *et al.*, 2011). Shera *et al.* (2017) also recorded *A. arizonensis* as a specific parasitoid of *P. solenopsis.* Nalini and Manickavasagam (2019) recorded *A. arizonensis* and *A. advena* as dominant parasitoids of *P. solenopsis* and *F. virgata* in Tamil Nadu. Similarly, a high rate of parasitisation on *F. virgata* by *A. advena* was noted by Ayyamperumal (2019) and Krishnamoorthy *et al.* (2021).

An encyrtid parasitoid *B. insularis* was recorded from three mealybugs *viz., F. virgata, P. solenopsis* and *P. jackbeardsleyi* from Kerala. Nalini and Manickavasagam (2016) recorded *B. insularis* as a parasitoid of *F. virgata* and *P. solenopsis* in Tamil Nadu. Similar observations were also made by Ayyamperumal (2019). The host-parasitoid association of *B. insularis* and *P. jackbeardsleyi* was recorded for the first time.

Two encyrtid parasitoids *viz., L. nigrocincta* and *L. tsukumiensis* were recorded from the mealybug *C. insolita,* of which the record of *L. tsukumiensis* on *C. insolita* was documented for the first time in the world. Nalini (2015) and Nalini and Manickavasagam (2016) reported *L. nigrocincta* as a parasitoid of *C. insolita* in Tamil Nadu.

The parasitoid, *P. unfasciativentris* belongs to the family Eriaporidae was observed on mealybugs such as *C. insolita, P. marginatus, F. virgata* and *P. solenopsis* in Kerala. Jhala *et al.* (2009) and Bharathi and Muthukrishnan (2017) documented *P. unfasciativentris* as the primary parasitoid of *P. solenopsis.* However, Ayyamperumal (2019), Torfi *et al.* (2020) and Chen *et al.* (2021) reported *P. unfasciativentris* as a parasitoid of *A. arizonensis* and a hyper parasitoid of *P. solenopsis.* The host-parasitoid associations of *P. unfasciativentris - C. insolita* and *P. unfasciativentris- P. marginatus* were recorded for the first time in the world.

M. comperei, which belongs to the family Eriaporidae, was also recorded from *P. solenopsis* in Kerala. However, it was recorded as a hyperparasitoid associated with *P. solenopsis* by Ruan *et al*. (2012) and Suroshe *et al.* (2013) whereas Padmanabhan (2017) and Chen *et al.* (2021) recorded it as a parasitoid of *P. solenopsis* from Kerala and China respectively.

Two species of parasitoids belongs to the superfamily Proctotrupoidea were found to be parasitizing on mealybugs *P. jackbeardsleyi* and *C. insolita* in Kerala. Yasnosh (2016) reported the members of the family Proctotrupoidea from mealybugs, *Pseudococcus* sp. and *Planococcus* sp.

Among the various mealybugs observed in Kerala, *P. solenopsis* was recorded with the maximum number of parasitoids followed by *C. insolita* and *F. virgata* (Fig.16). This may be due to the abundance of these mealybugs in the agroecosystems of Kerala.

The present study also identified six hyperparasitoids belongs to the family Encyrtidae which comprised of the genera *Cheiloneurus* and *Prochiloneurus.*

Among these, two species of *Chiloneurus* were recorded as new to the science. The genus *Prochiloneurus* is usually a hyperparasitoid of mealybugs and scale insects *via* parasitization on primary encyrtid parasitoids (Hayat, 2006). Wang *et al.* (2014) documented *Prochiloneurus* sp. as a parasitoid of *A. arizonensis* in China and Ayyamperumal (2019) recorded *Prochiloneurus* and *Chiloneurus* sp. as hyperparasitoids of *P. solenopsis* and *F. virgata* through *A. arizonensis* in India. The new hyperparasitoids were found as a menace to the natural biological control methods that kept the mealybug population under check.

5.1.7 Documentation of Ants Associated with Mealybugs

Ants are one of the most dominant components of the terrestrial ecosystem and were engaged in a myriad of interactions with other organisms especially honeydew-producing hemipterans.

A total of 14 species of ants belongs to the subfamilies Formicinae, Myrmicinae and Dolichoderinae were found to be in association with mealybugs in Kerala. Najitha (2016) recorded four species of ants belongs to the subfamilies Formicinae and Myrmicinae associated with the root mealybugs of pepper whereas Manjushree *et al.* (2019) recorded two species of ants belongs to the subfamilies Formicinae and Dolichoderinae associated with *D. brevipes* in Kerala. Sachin (2021) also recorded a total of seven ant species under the subfamilies Formicinae, Myrmicinae and Dolichoderinae associated with root mealybugs in Kerala.

The subfamily Formicinae (50 %) was the predominant one followed by Myrmicinae (36 %) and Dolichoderinae (14 %) (Fig.17). Similar observations were also made by Mitler and Douglas (2003) and Gowda *et al.* (2014). Among the mealybug attended ants recorded from Kerala, the highest diversity was noted with the genus *Camponotus* with four species followed by the genus *Solenopsis* with three species (Fig.18).

The ant-mealybug associations were usually considered as mutualistic as the ants provide shelter and aid in the dispersal of mealybugs whereas mealybugs

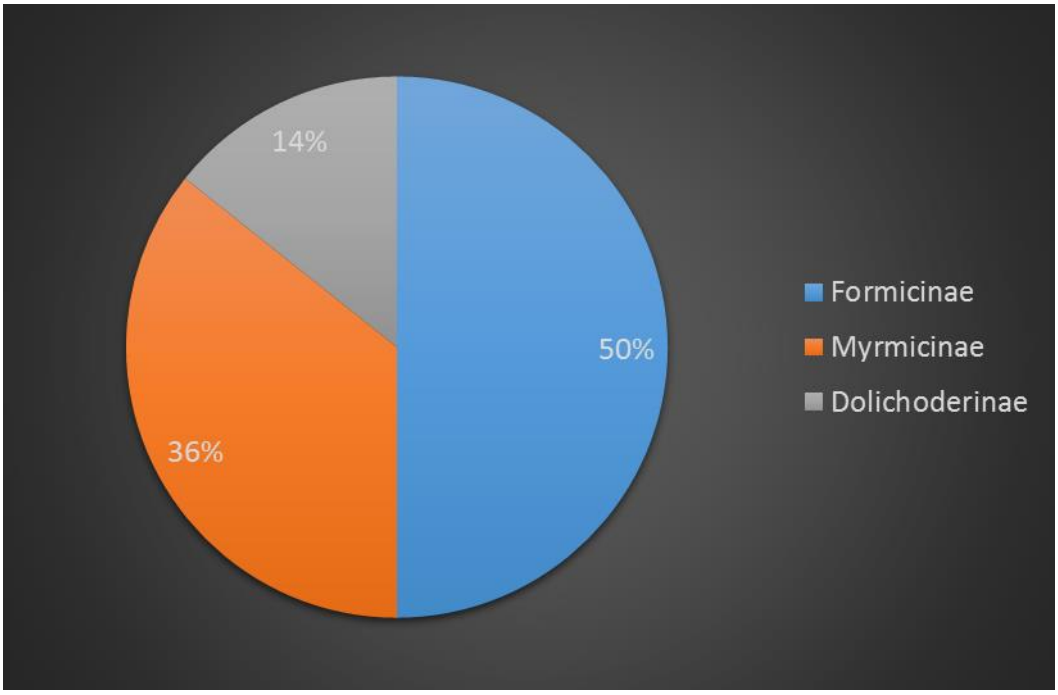


Fig. 17. Diversity of ants associated with mealybugs infesting solanaceous and cucurbitaceous vegetables - subfamily wise

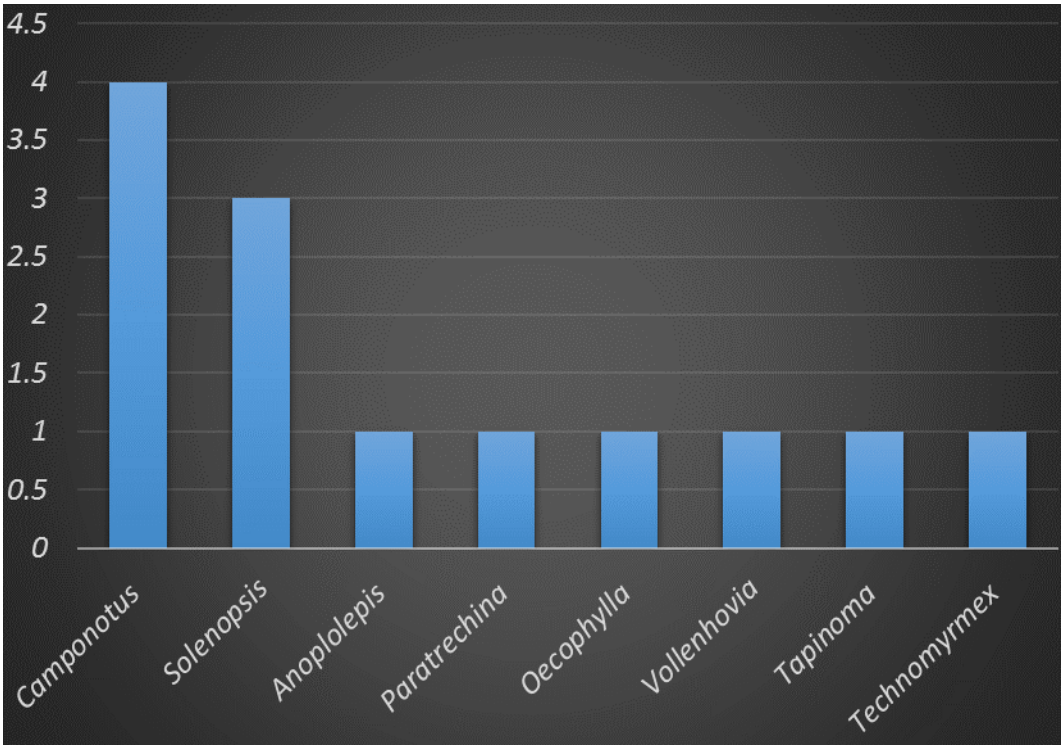


Fig. 18. Diversity of ants associated with mealybugs infesting solanaceous and cucurbitaceous vegetables - genus wise

provide honeydew to ants (Gutierrez *et al.,* 2008). Besides these, ants also provide protection from natural enemies thus enable the easy establishment of mealybug colonies (Mahimashanthi *et al*. 2014: Singh and Kumar, 2017; Xu *et al.* 2020).

Among the ant-mealybug interactions, the maximum number of associations were exhibited by the ant species, *A. gracilipes* which recorded interactions with four different mealybugs *viz., C. insolita, P. marginatus, P. citri* and *P. solenopsis* in Kerala. It was followed by *C. compressus, C. parius, C. barbatus, O. smaragdina, T. melanocephalum* and *T. albipes* (Fig.19). Fanani *et al.* (2020) reported that the mealybug attendant ants *viz., A. gracilipes* and *O. smaragdina* on cassava plants disrupted the efficiency of the parasitoid, *A. lopezi* infesting *P. manihoti.* Mohapatra *et al.* (2021) reported that *A. gracilipes* depends on *P. solenopsis* for carbohydrate and in turn protected the mealybug from predators.

Venkatesha (2018) reported that the genus *Camponotu*s exhibited aggressive behavior towards *C. montrouzieri* and *S. epius* predators in the mealybug colony and thus reduced the predation rate on mealybugs. The mealybug attended ants, *T. melanocephalum* improved the colony growth of *P. solenopsis* and also reduced the parasitization of *A. bambawalei* through aggressive confrontations and release of pygidial gland secretions (Liu *et al.,* 2020).

On comparing the associations of various mealybugs, the majority of interactions were made by the mealybug *C. insolita* with nine different ant species *viz. C. compressus, C. parius, C. barbatus, O. smaragdina, A. gracilipes, P. longicornis, Solenopsis* sp., *S. globularia* and *T. melanocephalum* (Fig. 20). However, Garcia- Morales *et al.* (2016) recorded three ant species *viz., A. gracilipes, Dolichoderus* sp. and *S. geminata* associated with *C. insolita.*

About 15 new ant-mealybug associations were recorded for the first time. The emergence of these new associations should be carefully addressed as they interfere with the balance of sustainable ecosystems.

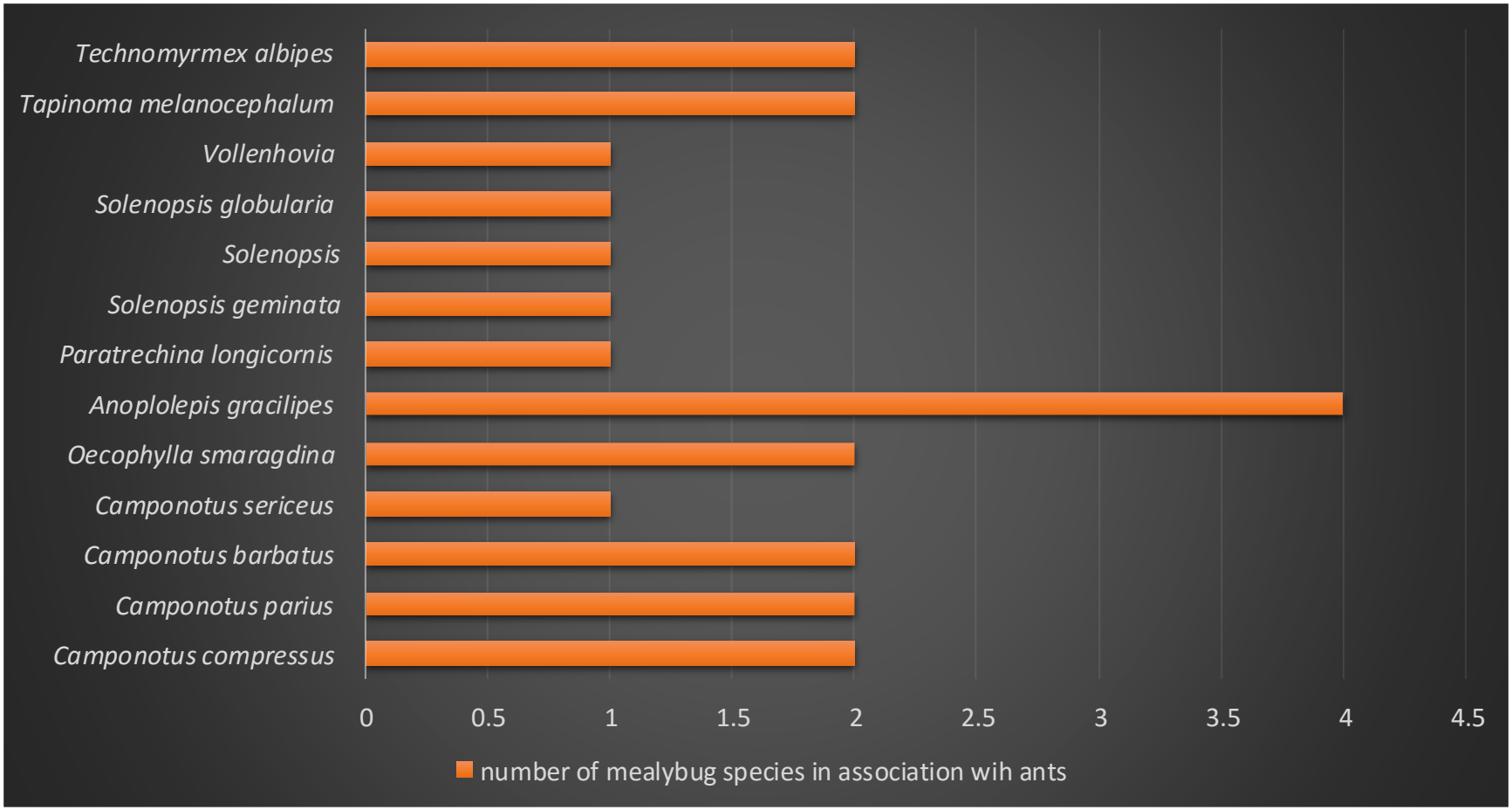


Fig. 19. Association of ants and mealybug species infesting solanaceous and cucurbitaceous vegetables in Kerala

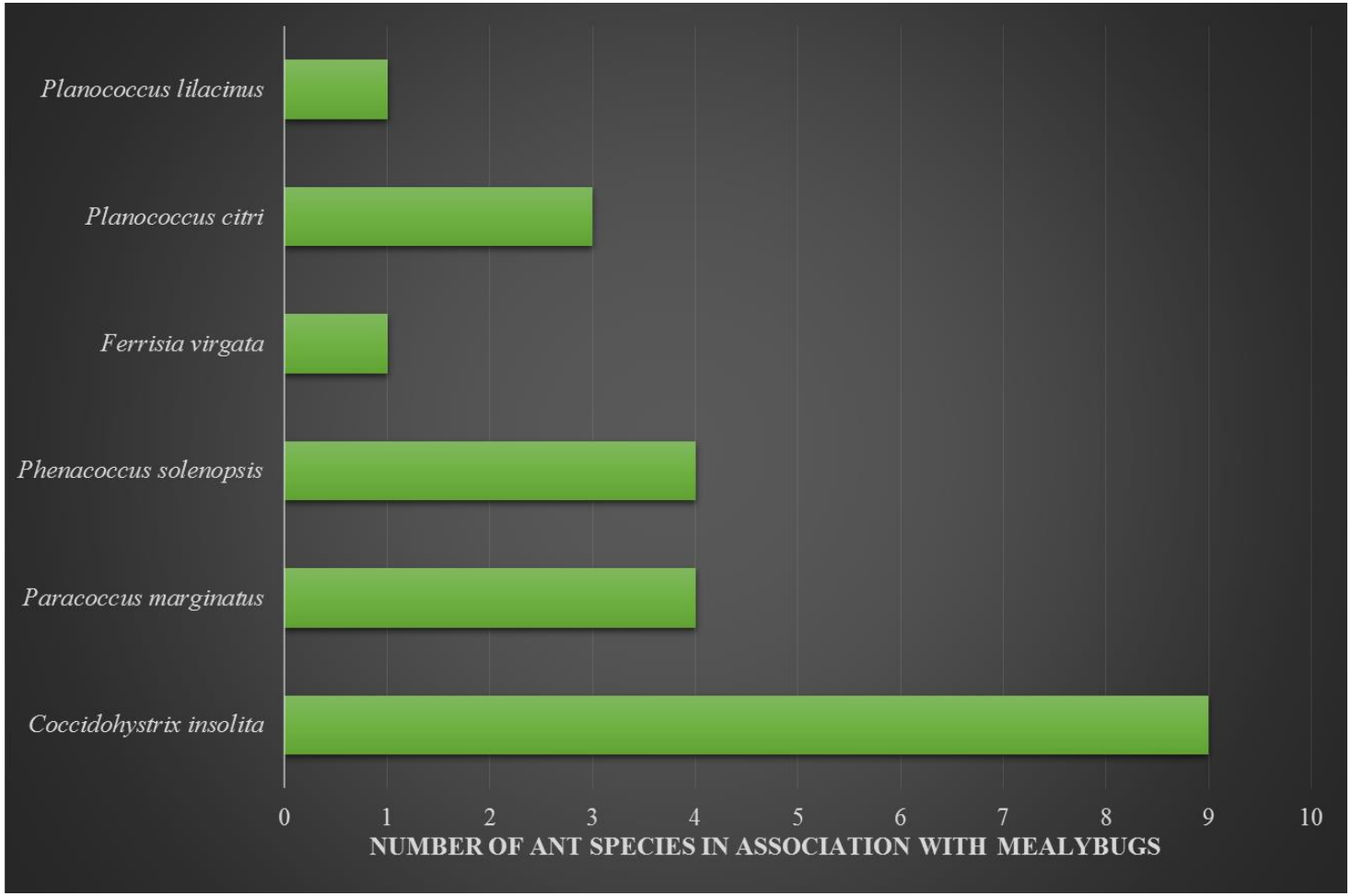


Fig. 20. Association of mealybugs infesting solanaceous and cucurbitaceous vegetables and ant species in Kerala.

5.2 MOLECULAR CHARACTERIZATION OF MEALYBUGS COLLECTED FROM BRINJAL AND PUMPKIN

The morphological identification of mealybugs is a tedious task due to the presence of cryptic species and phenotypic variations in the organism. In this scenario, the advent of molecular techniques aided in the easy and rapid identification of organisms. Integration of morphological and molecular approaches provided a reliable identification of mealybugs.

The homology of the sequence generated was checked with the database available at the NCBI and corresponding species were identified. The sequence generated for *P. solenopsis*, *M. hirsutus*, *P. jackbeardsleyi*, *P. lilacinus* and *F. virgata* showed significant homology with the corresponding sequences at NCBI database.

The species *P. citri* identified through morphological studies showed more similarity to *P. minor* in the NCBI database. The variation may be associated with the difficulty and complication involved with the exact identification of *P. citri* and *P. minor* as suggested by Rung *et al*. (2008). Sousa (2018) also reported that these species exhibits higher morphological similarity and often share common hosts that made the identification a tedious task.

The species *C. hirsutus* exhibited 95.48 per cent similarity to the sequence submitted as *P. citri* in the database and 94.24 per cent similarity to *C. hirsutus* accession. Williams (2004) reported that genus *Crisicoccus* and *Planococcus* exhibited close affinity and the morphological separation of these genera was a difficult task and also suggested that the genus *Crisicoccus* is in great need of molecular studies as he suspects that many of the species included in the genera were congeneric to the type species or not. So detailed and thorough studies were needed for solving this conflict.

The mealybug, *C. insolita* did not exhibit significant similarity with the available database and it may be due to the lack of availability of sequences to compare as limited studies were conducted on *C. insolita*.

The DNA barcoding considered as a decisive tool for precise, quick and reliable identification of insect species. The present study also generated illustrative barcodes for eight species of mealybugs.

5.3 MOLECULAR CHARACTERIZATION OF GUT ENDOSYMBIONTS OF *C. INSOLITA*

A study was conducted to identify the endosymbionts of the mealybug *C. insolita* as the endosymbionts played a vital role in determining the feeding behaviour of the mealybug. The endosymbionts of the mealybug *C. insolita* was identified for the first time. These organisms aid in the production of vitamins, digestion and development of resistance against synthetic pesticides (Douglas, 2009). So the basic knowledge on endosymbiont diversity of mealybug aid in a better understanding of the tritrophic interactions in the ecosystem and thus aid in devising a better pest management strategy.

The present study revealed a total of 15 phyla of endosymbionts in *C. insolita,* of which Proteobacteria was the predominant one followed by Euryarchaeota, Firmicutes, Bacteroidetes etc. The Phyla Proteobacteria were involved with the synthesis of essential amino acids for the growth and development which were lacking in the mealybug diet (Gatehouse *et al.,* 2012). Besides it played a vital role in pesticide detoxification (Kikuchi *et al.,* 2012). A study conducted by Szabo *et al.* (2017) reported that the major primary endosymbionts of the mealybugs belong to the group Proteobacteria. Similar results were also recorded by Lin *et al.* (2018). A study on characterization of endosymbionts of the mealybug *P. solenopsis* revealed that the major phylum observed was Proteobacteria followed by Firmicutes and Bacteroidetes (Padmanabhan *et al.,* 2019). An investigation on the endosymbionts of the mealybugs, *F. virgata* and *P. marginatus* revealed that Proteobacteria was the dominant category followed by Firmicutes and Actinobacteria (Jose *et al.,* 2020).

The present study revealed mainly three types of Proteobacteria in the mealybug *C. insolita viz.,* Gammaproteobacteria, Alphaproteobacteria and

Betaproteobacteria. Unlike other organisms, the endosymbionts of the mealybugs contain Betaproteobacteria as primary endosymbionts and Gammaproteobacteria as secondary endosymbionts (Munson *et al.,* 1992: Thao *et al.,* 2002). Iasur-Kruh *et al.* (2015) reported that the proteobacterial endosymbionts of mealybug, *Planococcus ficus* belongs to Betaproteobacteria and Gammaproteobacteria. Lin *et al.* (2018) reported that the mealybugs *viz.*, *D. neobrevipes*, *P. comstocki*, *P. solani*, *P. solenopsis* and *P. minor* harbored Gammaproteobacteria, Betaproteobacteria and Alphaproteobacteria. The most abundant endosymbionts of the mealybug *C. insolita* belongs to the class Gammaproteobacteria (41.36%). However, Padmanabhan *et al.* (2019) and Jose *et al.* (2020) recorded Betaproteobacteria as the dominant class of endosymbionts in *P. solenopsis*, *F. virgata* and *P. marginatus.*

The comparison on the relative abundance of endosymbionts of mealybugs at order level revealed that Pseudomonadales (40.55%) as the dominant group and the family level categorization showed that the maximum species composition was recorded with the Pseudomonadaceae (40.00) with *Pseudomonas* as the dominant genus. However, Lin *et al.* (2018) reported that *Candidatus tremblaya* as the dominant group of endosymbiont in the mealybugs *viz.*, *D. neobrevipes*, *P. comstocki*, *P. minor*, *P. solenopsis* and *P. solani.* Similar results were also recorded by Padmanabhan *et al.* (2019) in *P. solenopsis* and Jose *et al.* (2020) in *P. marginatus* and *F. virgata*. However, Megaladevi *et al.* (2020) reported that the metagenomic profile of mealybug species varied in different host plants and they pointed out that the genus *Pseudomonas* was recorded as the most abundant endosymbiont group in the mealybug *P. marginatus* infesting brinjal.

A total of 19 species of endosymbionts were recorded from the mealybug, *C. insolita*, of which the species *Pseudomonas alcaligenes* (24.37%) was the dominant one followed by an unclassified species belong to the genus *Pseudomonas* (15.02%). Gopal and Gupta (2002) reported that the presence of *P. alcaligenes* in the gut of rhinoceros grubs may limit the perpetuation of the virus, *Oryctes* sp. infesting rhinoceros grubs. *Pseudomonas* species recorded in

the mealybug, *P. marginatus* was recorded with proteolytic and Zinc solubilisation capacity (Megaladevi *et al.*, 2020). Ibrahim *et al.* (2021) recorded that the endosymbionts of the genus *Pseudomonas* observed in the mealybug *P. citri* was recorded to degrade organophosphate pesticides.

Lactobacillus is another group of bacterial endosymbiont associated with the gut of *C. insolita*. It was also recorded in the gut of *Apis* sp. and *Drosophila* sp. (Jeyaprakash *et al.,* 2003; Simhadri *et al.*, 2017). *Candidatus tremblaya* is also observed in the gut region of mealybug, *C. insolita.* Padmanabhan *et al.* (2019) recorded *Candidatus tremblaya princeps* as a major endosymbiont in the mealybug *P. solenopsis.* Similarly, it was also recorded in *P. marginatus* and it played a significant role in the synthesis of essential amino acids in insects (Megaladevi *et al.,* 2020).

The endosymbionts play a crucial role in survival and host range of organism, thus a thorough knowledge on these organisms led to a better understanding on the different trophic level interactions in the ecosystem

5.4. TRI-TROPHIC INTERACTION

5.4.1 Interaction in Brinjal Ecosystem Mediated by Mealybug, *C. insolita* and Its Natural Enemies

The dynamics of tritrophic interactions in an ecosystem may vary with host plant species or genotypes of host plants and a thorough understanding of these interactions are of utmost importance (Hare, 2002). In this context, an experiment was conducted to identify the interactions between different brinjal genotypes, mealybug *C. insolita* and its natural enemies at Instructional Farm Vellayani.

Out of the ten cultivars, the lowest mean population of mealybugs was observed in Pusa Uttam (38.58) which was statistically on par with Pusa Purple Long (41.67). The hybrid cultivar, Udit recorded the highest number of mealybugs (127.58) which was statistically on par with Pink Long (116.75) and Haritha (115.50) (Fig. 21). The lowest mean per cent leaf infestation was recorded

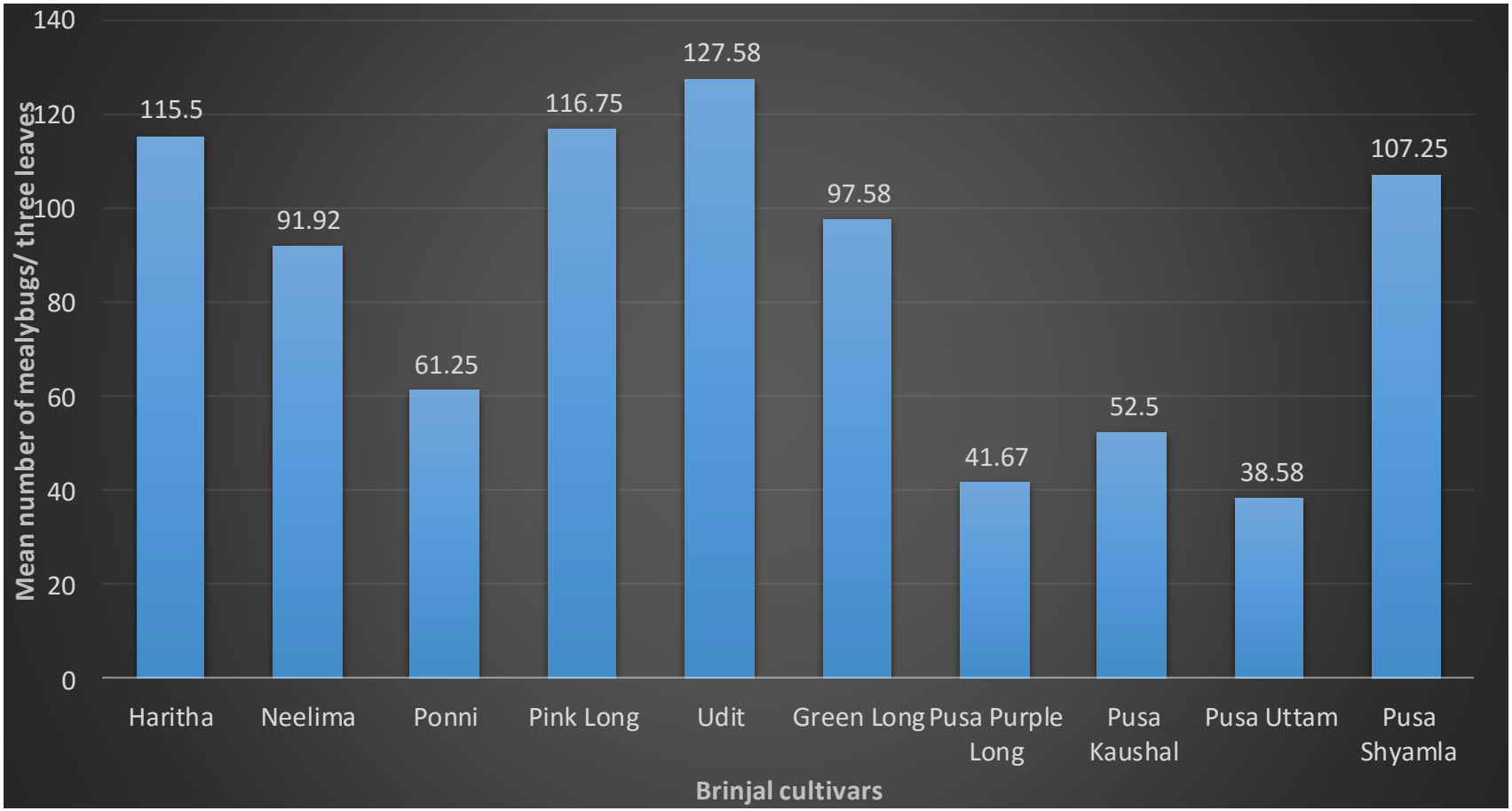


Fig. 21. Mean population of *Coccidohystrx insolita* in different brinjal cultivars

from Pusa Purple Long (8.96) which was statistically on par with Pusa Uttam (13.25) whereas the hybrid cultivar, Udit was severely damaged by the mealybugs with a mean per cent leaf infestation of 64.38 which was statistically superior to all other cultivars. The second highest infestation was noted with the cultivar, Haritha with a mean per cent leaf damage of 50.22 which was statistically different from all other cultivars. It was followed by the cultivar Pink Long, Green Long, Neelima and Pusa Shymala with mean per cent leaf damage of 34.80, 32.49, 28.94 and 28.65 respectively (Fig.22).

The ten brinjal cultivars were categorized into groups based on mean per cent leaf infestation caused by *C. insolita*. None of the cultivars was recorded to be immune to brinjal mealybug, *C. insolita*. Among the tested cultivars, Pusa Purple Long was recorded under the group resistant whereas Pusa Uttam and Ponni were included in the group moderately resistant. The cultivar Pusa Purple long was recorded to be tolerant to the jassid *Amrasca devastans* and the susceptibility level of brinjal cultivars varied according to the nutritional value of the cultivar (Gaikwad *et al*., 1991; Kalra, 2004). Similarly, Naqvi (2005) and Bilal *et al.* (2017) also reported that Pusa Purple Long was less susceptible to sucking pests owing to the morphological peculiarities of the cultivar.

The cultivars *viz.,* Pusa Kaushal, Pusa Shyamla and Neelima were contained in the group moderately susceptible while Pink Long (34.80) and Green Long (32.49) were designated under the susceptible category. The cultivars such as Udit and Haritha were included under the highly susceptible group based on the mean per cent leaf infestation caused by *C. insolita.* A study conducted by Reddy and Srinivasa (2001) recorded that hybrid Green Long was more susceptible to the sucking pest infestation compared to other tested brinjal cultivars. Malini *et al.* (2013) reported that the cultivar Haritha was highly susceptible to the jassid *A. biguttula biguttula* than other tested brinjal accessions in the field. The susceptibility of the cultivar Green Long was also pointed out by Bilal *et al.* (2017) as it harbored the second-highest nymphal (0.31)) and adult (0.46) population of jassids per leaf among the screened brinjal cultivars.

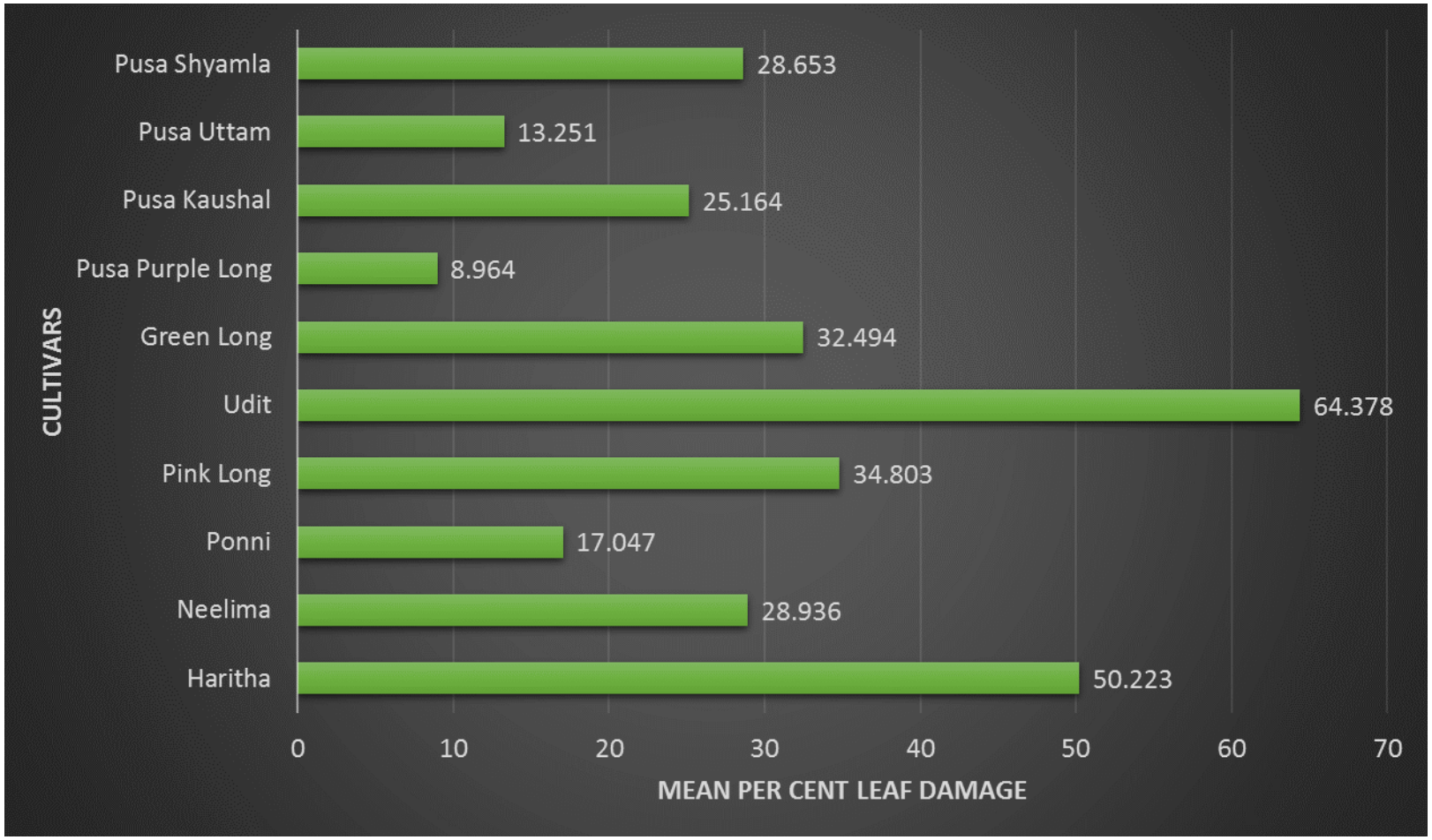


Fig. 22. Mean percentage leaf damage caused by *Coccidohystrix insolita* in different brinjal cultivars.

The cultivar, Haritha attracted the highest population of natural enemies (3.92) which was statistically superior to all other cultivars. The second highest population of natural enemies were recorded in the cultivar, Udit which was statistically on par with Pusa Shymala, Pusa Kaushal, Pink Long, Neelima and Pusa Purple Long. The cultivar Ponni attracted the lowest population of natural enemies (0.75) which was statistically on par with Green Long (0.92) and Pusa Uttam (0.92) (Fig.23)

On comparing the mean population of spiders in different cultivars during the entire crop period revealed that the highest was recorded in the cultivar Pusa Purple Long (1.42) which was statistically on par with Ponni, Haritha, Pink Long, Green Long and Pusa Shymala whereas the lowest was recorded on Pusa Kaushal (0.17) which was statistically on par with Pusa Uttam, Udit and Neelima (Fig.24). Elanchezhyan *et al.* (2008) reported that certain cultivars of brinjal harbors a high population of natural enemies like coccinellids, syrphids and spiders and it might be due to the variation in volatile emission from the different plant genotype. Ayub *et al.* (2020) reported that the population of natural enemies such as *C. septempunctata* and *C. carnea* were maximum in the brinjal cultivar Shamli hybrid and suggested that the plant morphological factors may be responsible for the preference shown by the natural enemies.

5.4.2 Biophysical Parameters Mediating Tri-trophic Interaction in Brinjal

The biophysical parameters of the host plant played a crucial role in determining the trophic level interactions in an ecosystem.

Among the tested cultivars, the highest trichome density was recorded with the cultivar, Udit. The lowest trichome density was noted in the cultivar Pusa Kaushal which was statistically on par with Pusa Purple Long. The cultivar Ponni was recorded with the highest leaf thickness of 0.29 mm which was statistically on par with Neelima and Pusa Uttam whereas the lowest leaf

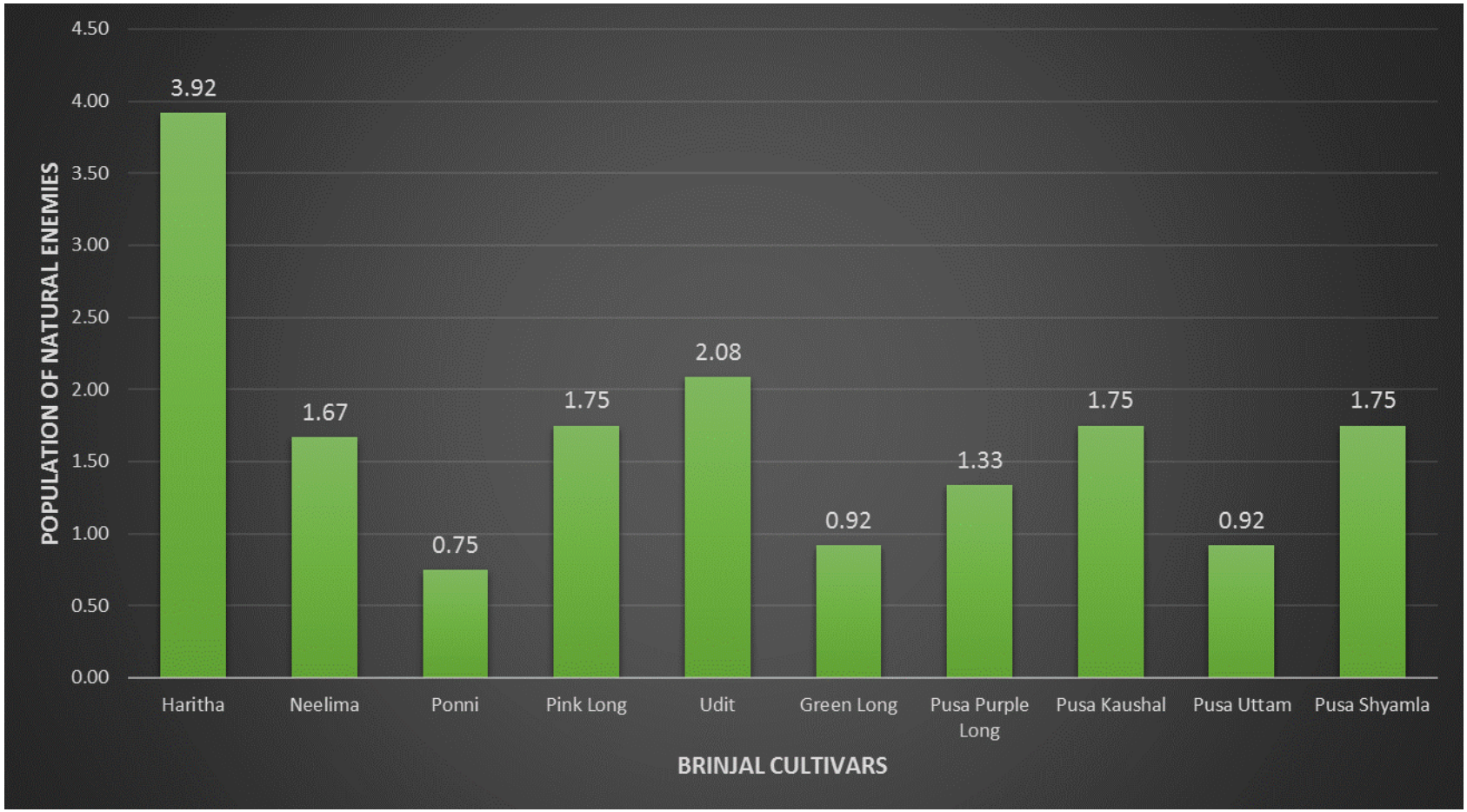


Fig. 23. Mean population of natural enemies of *Coccidohystrix insolita* in different cultivars of brinjal.

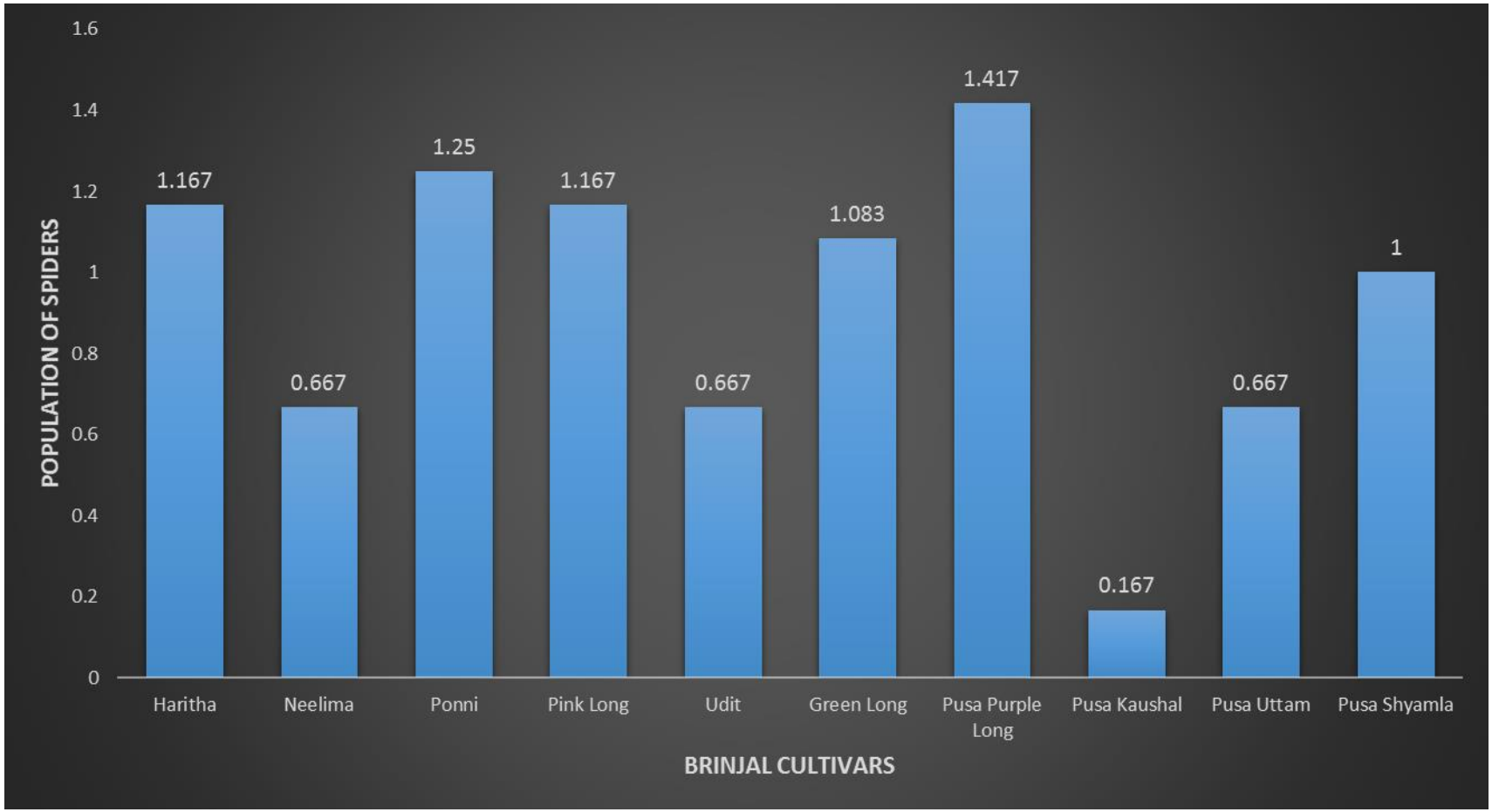


Fig. 24. Mean population of spiders in different cultivars of brinjal.

thickness was noted in cultivar Pusa Kaushal which was statistically on par with Udit.

The highest length-width ratio of leaf was noted with the cultivar Udit (1.97) which was statistically on par with Pusa Shymala (1.94) while the lowest length-width ratio was observed with the cultivar Pusa Uttam (1.32). The cultivar Pink Long was recorded with the highest number of branches plant^{-1} (13.33) which was statistically on par with the cultivar Pusa Shymala (13.33) and Neelima (11.00). The cultivar Pusa Uttam (2.33) was noted with the lowest number of branches plant^{-1} which was statistically on par with Pusa Purple Long (4.00). The cultivar Neelima (110.33 cm) was recorded with the highest plant height which was statistically on par with Green Long (109.33 cm) and Ponni (106.00 cm). The cultivar**,** Udit wad noted with the lowest plant height of 62.67 cm which was statistically on par with Haritha (65.33).

Correlation and regression studies on the mean population of mealy bugs with biophysical parameters were carried out and the regression equation obtained was $Y = 0.099\ X_1 - 124.602\ X_2 - 57.811\ X_3 + 7.784\ X_4 - 0.856\ X_5 + 140.088$. The correlation studies revealed that trichome density exhibited a significant positive correlation with the mean population of mealybugs. Johnson- Cicalese *et al.* (1998) pointed out that leaf pubescence exhibited a positive correlation with the population of mealybugs *Tridiscus* sp. and *Trionymus* sp. in buffalo brass genotypes and also suggested that the trichomes provide a foothold for first instar nymphs. Johnson- Cicalese *et al.* (2011) conducted scanning electron microscopic studies and revealed that early instars can easily move through the trichomes. The waxy materials produced by the mealybugs entangled with the trichomes and thereby modify the structure of leaf surface that provide better protection to ovisac and young ones. Similarly, a significant positive correlation was recorded with trichome density and population of mealybug *P. solenopsis* in different host plants and *M. hirsutus* in mulberry (Shahid *et al.,* 2012; Mahimasanthi *et al.,* 2014).

Leaf thickness exerted a non-significant negative correlation with the population of *C. insolita.* The increase in leaf thickness may interfere with the

feeding of mealybugs and thus act as a hindrance to population establishment in resistant cultivars. Shahid *et al.* (2012) reported that *P. solenopsis* population showed a non-significant negative correlation with leaf lamina thickness in cotton. However, Shahid (2016) reported that leaf thickness exerted a significant positive correlation with population of mealybugs in different host plants. Hasanuzzaman *et al.* (2016) and Khan *et al.* (2018) reported that the susceptibility of brinjal cultivars to whitefly, *B. tabaci* increased with the reduction in leaf thickness.

Correlation analysis disclosed that length width ratio of leaf and number of branches plant^{-1} exhibited a significant positive correlation with mean population of mealybugs whereas plant height showed a non-significant negative correlation. However, Ali *et al*. (2016) reported that plant height and number of branches showed a non-significant positive correlation with jassids in brinjal. An experiment conducted by Javed *et al*. (2016) and Ramzan *et al.* (2020) revealed that plant height showed a positive correlation with sucking pests in cotton and jassids in brinjal respectively.

Correlation and regression studies on mean population of natural enemies with biophysical parameters were carried out and the regression equation obtained was $Y= 1.430\ X_2 - 4.099\ X_3 + 0.202\ X_4 - 0.076\ X_5 +12.687$. The plant height exhibited a significant negative correlation whereas leaf thickness and number of branches plant^{-1} revealed a non-significant negative correlation with mean population of natural enemies. As the plant height and number of branches increases, it may interfere with the movement and host searching capacity of natural enemies. Similar results were also corroborated by Cloyd and Sadof (2000) in citrus mealybug *P. citri* and its parasitoid *L. dactylopii*. Garcia and O' Neil (2000) and Zhu (2016) also recorded that plant height showed a negative correlation with the population of *C. montrouzieri* predating on *P. citri*.

The present study revealed that trichome density and length width ratio of leaf showed a positive non-significant correlation with mean population of natural enemies. Conversely, Kennedy (2003) reported that trichome density hindered the movement and oviposition of natural enemies. However, Bjorkman and Ahrne

(2005) reported that higher trichome density may induce the natural enemy for a thorough search in the plant and thus led to a higher encounter rate with the prey. Al-Zyoud *et al*. (2005) reported that coccinellid predator, *Serangium* sp. showed more attractiveness towards hairy plant cultivars as it may reduce the cannibalism rate in larval stages.

5.4.3 Biochemical Parameters Mediating Tri-trophic Interaction in Brinjal

Biochemical parameters play a major role in conferring resistance to insect pests of crops and thus interfere with the population of natural enemies.

Phenols are one of the most important secondary metabolites present in the plants which act as feeding deterrents or growth retarding compounds of insect herbivores. The cultivar, Pusa Uttam possessed the highest quantity of total phenol (4.62 mg $g^{-1)}$) which was significantly superior to other cultivars. The lowest total phenol content was recorded in the cultivar, Udit (1.14 mg g^{-1}). The highest protein content was recorded with Pusa Kaushal (12.06 mg g^{-1}) which was statistically on par with Neelima (10.43 mg g^{-1}) whereas the lowest protein content was recorded with the cultivar Pink Long (6.36 mg g^{-1}). The cultivar, Pusa Purple Long showed the highest reducing sugar (2.87 mg g^{-1}) whereas the concentration of reducing sugars was found to be the lowest in Pusa Uttam (1.19 mg g^{-1}).

The photosynthetic pigments in the plants also played a significant role in host plant-pest interactions. The chlorophyll content was found to be highest in the cultivar Udit (2.84 mg g^{-1}) which was statistically superior to all other cultivars. The lowest chlorophyll content was noted with the cultivar Pusa Uttam (1.55 mg g^{-1}). The highest carotenoid content was noted with the cultivar Pusa Uttam (0.96 mg g^{-1}). The lowest carotenoid content was recorded with Green Long (0.59 mg g^{-1}).

The total phenol content exhibited a significant negative correlation with the mean population of mealybugs. Phenolics are defensive compounds present in plants that inhibited the behavior, feeding, growth, metabolism and development

of insect pests. (Lattanzio *et al.,* 2006; Alba *et al.,* 2015; Tripathi *et al.,* 2019). Smitha (2007) recorded that root mealybugs exhibited non-preference towards the banana cultivars with a higher concentration of total phenols. Janaki and Suresh (2012) elucidated that the higher phenolic content in leaves imparted resistance to the papaya mealybug, *P. marginatus* in brinjal. Likewise, Mahimasanthi *et al*. (2014) and Amala (2015) also recorded a negative correlation of total phenols with the population of mealybug, *M. hirsutus* in mulberry and grapevines respectively.

The correlation analysis revealed that protein content exhibited a non-significant negative correlation with mean population of mealybugs. Similar results were corroborated by Mahimasanthi *et al*. (2014). But these results are in conformity with Amala (2015) who recorded a positive significant correlation of *M. hirsutus* with total protein concentration in grapevine cultivars. Azouz *et al.,* (2014) and Khan *et al.* (2018) also recorded a positive significant correlation of protein content with the population of sucking pests and whiteflies in brinjal respectively.

The reducing sugar content in different brinjal cultivars showed a non-significant positive correlation with the mealybug population. The higher sugar content in leaves acts as a phagostimulant which promote the insect herbivory in susceptible cultivars (Kalode and Pant, 1967). Eid *et al.* (2011) and Yakoub (2012) recorded that mean population of mealybugs, *S. sacchari* showed a positive correlation with reducing sugar content in different sugarcane cultivars. Nisha and Kennedy (2017) recorded that *P. marginatus* exhibited a positive association with sugar content in the host plants as the direct availability of reducing sugars provided a better growth environment for mealybugs.

The total chlorophyll content showed a positive significant correlation with the mean population of mealybugs in brinjal cultivars. These results are supported by the findings of Janaki and Suresh (2012); Amala (2015) and Shahid (2016). The higher levels of chlorophyll content in leaves may be associated with the higher palatability of tissues as it was positively correlated with the soluble

nitrogen in the plant tissues. Since nitrogen is one of the limiting factors for development and oviposition in insects, herbivores preferred plants with higher chlorophyll content (Sousa- Souto *et al.,* 2018).

Carotenoids are ubiquitous organic molecules and their role in mediating multi-trophic interactions in an ecosystem is only beginning to be understood. Carotenoids are one of the most important photosynthetic pigments which reduced the negative impacts of photo-oxidation on chlorophyll and thylakoid membrane. Besides, the differences in carotenoid compounds in host plants may affect the behavior and survival of insect pests (Tefler *et al.,* 2008). Carotenoid content exhibited a non-significant negative correlation with mean population of mealybugs. This finding is consistent with Helmi and Rashwan (2015) and Golan *et al.* (2015).

The correlation between biochemical parameters of brinjal cultivars with the mean population of natural enemies was carried out and the regression equation obtained was $Y=-0.308\ X_1 +1.744\ X_2 -0.160\ X_3 + 0.892\ X_4 - 0.496\ X_5 + 0.013$. The phenol content exhibited a non-significant negative correlation whereas total protein content and reducing sugar exhibited a non-significant positive correlation with mean population of natural enemies. As the phenol content increases, it may adversely affect the population of herbivores which in turn led to a reduction in natural enemy population. Burger *et al.* (2005) also reported that the nutritional quality of host insect may depend on the plant species and its physiological status which in turn affect the natural enemies. The higher levels of protein and sugar in plants increase the palatability of tissues which led to the better establishment of mealybugs and thus increased the attraction of natural enemies. These results are in partial conformity with Khan *et al.* (2014) who reported that the total protein content showed a negative correlation whereas sugar content exhibited a positive correlation with the population of natural enemies *viz., C. septempunctata, C. carnea* and *E. balteatus* in brinjal.

The total chlorophyll content exhibited a non-significant positive correlation whereas carotenoid content showed a non-significant negative

correlation with mean population of natural enemies. The photosynthetic pigments usually act as a visual cue for natural enemies which aid in host habitat location. Similar results were also reported by Blackmer and Cross (2001).

5.4.4 Info- Chemical Mediated Tri-trophic Interaction in Brinjal, Mealybug and Natural Enemies

Info-chemicals played a vital role in maintaining tri-trophic interactions *via* mediating interspecific communication in an ecosystem. Info-chemicals are regarded as an indirect phytochemical defense that assists plants in protecting themselves from herbivore damage (Gatehouse, 2002).

The info-chemical mediated interactions in brinjal, mealybug and its natural enemies were studied by using a multi-armed olfactometer and Y-shaped olfactometer assay.

5.4.4.1 *Response of C. zastrowi sillemi to Synomonal Compounds of Brinjal*

The preference of natural enemy, *C. zastrowi sillemi* towards the synomonal compounds extracted from the brinjal cultivars was evaluated in a multi-armed olfactometer and the results revealed that the cultivar Udit attracted the highest number of natural enemies (2.89) while the cultivar Pusa Uttam attracted the lowest number (0.28). The variation in preference shown by the natural enemies may be related to the difference in volatile compounds emanating from the host cultivar. Hanumantharaya (2006) conducted a six-armed olfactometer study to evaluate the response of the predator, *C. carnea* towards the synomonal extracts of cotton and sunflower genotypes revealed that the preference shown by the predator towards the cultivar DHH-543 and KBSH-1 was due to the difference in the volatile profile of the cultivars. Similarly, Trang (2008) also reported that the parasitoid *Apanteles angaleti* Mues exhibited a higher preference towards the synomonal compounds of cotton genotype RS 2013 in a six-armed olfactometer study as the volatile profile of the cultivar made it more attractive to the parasitoid compared to other genotypes. Kumar *et al.* (2017) reported that the synomones of the sugarcane cultivar, CO- 0238 attracted

the highest number of parasitoid, *Cotesia* sp. and they suggested that the long-range cues emanating from the infested host plants played a significant role in guiding the parasitoids to the host plant.

The GC-MS analysis of the synomonal compounds of the cultivar, Udit showed eleven compounds whereas only five compounds were obtained from the cultivar Pusa Uttam. The higher number of volatile compounds in the cultivar Udit may attract a higher number of natural enemies compared to the cultivar Pusa Uttam with fewer volatile compounds. Rathika and Nalini (2011) reported that the leaf folder susceptible rice cultivar TN 1, attracted the highest number of braconid parasitoids and recorded with eight volatile compounds whereas the resistant cultivar Ptb 33, attracted less number of parasitoids and was noted with 5 compounds.

The compounds *viz.*, diisooctyl phthalate, hexadecane, dodecane and heptadecane were common in the synomonal extracts of both cultivars, but the concentration of these compounds varied in two cultivars. The most dominant compound diisooctyl phthalate was found to be higher in Pusa Uttam (45.60%) than that of Udit (33.25%). Diisooctyl phthalate is a phthalate ester observed in the volatile profile of rice cultivar TN1, *Dendrobium* sp., *Celtis* sp., *Pistia* sp., etc. and recorded with anti-microbial properties. (Sangeetha and Vijayalakshmi (2011); Rathika and Nalini (2011); Tyagi and Agarwal (2017); Hu *et al.* (2020); Wei and Guo (2020). The role of diisooctyl phthalate in mediating tri-trophic interactions are meagre.

The compounds *viz.*, hexadecane and heptadecane were found to be the highest in Udit compared to Pusa Uttam whereas dodecane was highest in Pusa Uttam. An experiment conducted by Bhagat and Bakthavatsalam (2012) revealed that the compounds *viz.*, hexadecane and heptadecane were responsible for the higher preference of parasitoid *T. japonicum* to the rice cultivar Kadamba. Nishintha *et al.* (2019) reported that Dodcanae is an important volatile compound in *Scirpophaga incertulas* (Walker) damaged rice plants and were responsible for the attraction of natural enemies. Xu *et al.* (2021) conducted a reverse chemical

ecology experiment, based on the physiological function of odorant-binding proteins (OBPs) revealed that dodecane was one of the important compounds that elicited a positive response in parasitoid *A. bambawalei* infesting *P. solenopsis* in cotton.

The GC-MS profile of cultivar Udit revealed 7 more compounds *viz.*, beta-Eudesmene (9.53%), eremophilene (5.19%), decane, 2,3,5,8-tetramethyl (4.60%), Octane, 3-ethyl-2,7-dimethyl (4.00%), N-(trifluoroacetyl)-N,O,O',O"-tetrakis (trimethylsilyl) norepinephrine (2.40%), nonane, 3,7-dimethyl (2.36 %) and oxalic acid, allyl decyl ester (1.27%) which were absent in the cultivar Pusa Uttam. The majority of volatile compounds released as a result of insect herbivory are terpenoids *viz.,* monoterpenes and sesquiterpenes that lure natural enemies to plants (Degenhardt *et al.,* 2003). Troncoso *et al.* (2011) reported that the sesquiterpene, eremophilene was recorded in the plant *Eucalyptus globulus* (Labill) infested by blue gum psyllid as a defense response in plants. Sahla and Pushpalatha (2020) reported that the GC-MS analysis of essential oils of *Melaleuca leucadendron* L. revealed the sesquiterpene compound beta-Eudesmene which was recorded with insecticidal and fumigant properties against *Callosobruchus maculatus* (Fab.). The role of this compound in tritrophic interactions are meagre.

Long-chain hydrocarbons are involved in tritrophic interactions in an ecosystem *via* acting as a chemical cue for natural enemies (Trang and Dey, 2013). A study conducted by Pavviya *et al.* (2016) revealed that the saturated hydrocarbons *viz.,* decane, hexadecanoic acid, pentacosane, cyclohexanol, tricosane and 3-hexanol were responsible for the natural enemy attraction in rice stem borer damaged rice plants. Kumar (2019) also reported that the long chain hydrocarbons *viz.,* decane, 2,3,5,8-tetramethyl, decane, 2,3,5,8-tetramethyl, decane, 2,3,5,8-tetramethyl, Nonane-2- methyl, dodecane etc. were included in the herbivore-induced plant volatile profile of okra plant infested by bhindi fruit and shoot borer and were responsible for the attraction of parasitoid *T. chilonis*.

N-(Trifluoroacetyl)-N,O,O',O"-tetrakis (trimethylsilyl) norepinephrine is another compound observed in the volatile profile of the cultivar, Udit which was recorded with molluscicidal activity in *Juniper* sp. (Ghaly *et al.,* 2016). The role of these compounds in tri-trophic interactions is not available to support the present findings.

Oxalic acid, allyl decyl ester is another compound that belongs to the group esters present in the cultivar Udit and usually, esters were involved in mediating communication between host plant and natural enemies. Wonorahardjo *et al.* (2018) reported that long-chain hydrocarbons and esters act as chemical cues for parasitoid, *Anangrus nilaparvateae* for host habitat finding.

The cultivar Pusa Uttam was recorded with the compound, tetradecane (21.94%) which was not present in the volatile profile of the cultivar Udit. Roman-Ruiz *et al.* (2012) reported n-tetradecane as a repellant to the parasitoid *Prorops* sp. infesting coffee berry borer. However, Silveira *et al.* (2018) reported that tetradecane released from melon plants due to whitefly infestation led to the attraction of parasitoid *Encarsia desantisi* Viggiani.

The differences in compounds and their concentration in the volatile profile of the cultivars may lead to the highest preference of *C. zastrowii sillemi* to Udit and the lowest preference to Pusa Uttam.

Based on the results of multi-armed olfactometer, another experiment was devised to identify the relative response of *C. zastrowi sillemi* adults to the synomonal extracts of healthy and mealybug infested brinjal cultivar Udit (most preferred) in a Y- tube olfactometer. The results revealed that *C. zastrowi sillemi* adults showed more preference towards the synomonal compounds of mealybug infested plants than that of healthy synomonal extracts. The relatively high preference of natural enemies towards mealybug infested plant synomonal extracts may due to the presence of more volatile compounds in it compared to the healthy plant synomones. As a result of insect herbivory, a cascade of events take place in the plants which ultimately led to the higher production of plant volatiles

that act as a reliable long-distance cue for natural enemies. The present findings are in consonance with Bertschy *et al.* (2001); Le and Makosso (2001) and Bue *et al.* (2004). Xie *et al.* (2004) demonstrated similar results in a Y tube olfactometer experiment using synomonal extracts of *P. azaleae* infested Bunge prickly ash and healthy plants using the predator *H. axyridis.* Jagdish (2008) and Xiu *et al.* (2019) also demonstrated that coccinellid predators showed a higher preference towards the aphid infested plant synomonal compounds compared to healthy plant odors.

On comparing the volatile profile of synomones of mealybug infested and healthy plants revealed that the synomonal extracts of the mealybug infested cultivar Udit comprised of 11 compounds whereas healthy plant synomonal extracts contained nine compounds. Gautam *et al*. (2010) reported that mealybug, *P. solenopsis* infested cotton plant was recorded with 18 more synomonal compounds than that of a healthy plant. Kamala *et al.* (2017) reported the synomone of blossom midge damaged jasmine with 34 saturated hydrocarbons whereas synomones of the healthy plant comprised of 24 hydrocarbons and also suggested that the variation in hydrocarbon profile elicited a differential response in natural enemies. These results are also in consonance with Paviyya *et al*. (2018).

The compounds *viz.,* hexadecane, heptadecane and dodecane were common in healthy and mealybug infested plant synomones. However, mealybug infested plant volatiles recorded a higher concentration of these compounds than healthy plant synomones. Similar observations were made by Xie *et al.* (2004) in a tritrophic system involving *P. azaleae* and its predator, *H. axyridis in* Bunge prickly ash. Ahmed *et al.* (2021) also reported that aphid infested broccoli plants emitted a higher concentration of volatile organic carbons than un-infested plants.

The healthy plant synomonal extracts of the cultivar Udit gave six more compounds, of which tetratetracontane was noted as the most dominant compound. The compounds *viz.,* tetradecane, hexatriacontane, 2-methyloctacosane, thymine glycol and 13-hexyloxacyclotridecan-2-one were also observed in the healthy plant synomonal extracts of Udit. The compound

tetratetracontane was recorded from the volatile profile of healthy okra (Kumar *et al.,* 2017) whereas hexatriacontane was recorded from mechanically damaged okra plants (Kumar, 2019). The role of methyl octacosane, thymine glycol and 13-hexyloxacyclotridecan-2-one in mediating tritrophic interactions are meagre. The variation in the volatile profile of healthy and mealybug infested plants may lead to the differential response of natural enemies to the synomonal compounds.

5.4.4.2 ***Response of C. zastrowi sillemi to Kairomonal Compounds of C. insolita.***

The relative response of *C. zastrowi sillemi* towards the kairomonal compounds of *C. insolita* was evaluated in a Y tube olfactometer and the results revealed that the highest mean number of adult lacewings were attracted to the kairomonal compounds of mealybug compared to control. The higher preference of natural enemies towards the kairomonal compounds may be due to the volatiles present in it which act as an olfactory cue for natural enemies. Similar findings were corroborated by Calatayud *et al.* (2001) as they reported that the parasitoids *viz., A. coccois* and *A. vexans* exhibited a higher preference towards the kairomonal extracts of mealybug *P. herreni* compared to control. Gautam *et al.* (2010) also elucidated similar results in a Y tube olfactometer using kairomonal extracts of *P. solenopsis* and the predator *C. carnea*. Fand *et al.* (2020) also reported that the bacterial volatiles of the mealybug honeydew acts as a kairomone source to the parasitoid *A. dactylopii*.

The kairomonal extracts of the mealybug comprised of seven compounds, of which 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris (trimethylsiloxy) tetrasiloxane was recorded as the dominant compound. The compounds *viz.*, cyclooctasiloxane hexadecamethyl, squalene, dodecane, 2-bromo dodecane, sulfurous acid, hexyl octyl ester and undecane 4,7-dimethyl were also recorded as the volatile compounds in the mealybug kairomonal extract. The information on role of 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris (trimethylsiloxy) tetrasiloxane, cyclooctasiloxane hexadecamethyl and sulfurous acid, hexyl octyl ester in tritrophic interactions were not available.

The tri-terpene compound squalene is recorded as an ovipositional cue for parasitoids of apple leaf miner (Dutton *et al.,* 2002) and it was also recorded as a potential semiochemical that attracted the predator *Chrysopa* sp. in apple orchards (Jones *et al.,* 2011). It was also recorded as an attractant volatile cue for coffee bean weevil (Yang *et al.,* 2017).

The long-chain alkenes *viz.,* dodecane, 2-bromo dodecane and undecane 4,7-dimethyl were also involved in natural enemy attraction to host habitat. Han and Chen (2002) reported that undecane present in the aphid body wash elicited a favorable response in *Chrysopa* sp. and *C. septempunctata*. Fand *et al.* (2020) reported that dodecane present in the bacterial volatiles of mealybug honeydew elicited a positive response in the parasitoid *A. dactylopii*. The difference in composition of hydrocarbons in volatile blend may determine the selectivity of natural enemies towards the preferred host.

Summary

6. SUMMARY

Mealybug, the hard to kill organism, is one of the economically important pest causing alarming damage in crops worldwide. Despite being a small delicate soft bodied insect, the management of mealybug is an arduous task due to the waxy coating over the body, cryptic habitat and spatial distribution pattern. Besides, the injudicious application of chemicals led to disruption of natural enemy population and development of resistance (Mani and Shivaraju, 2016). In this scenario, a better perception of tritrophic interactions in the ecosystem and manipulation of theses interactions led to the development of a sustainable ecologically safe pest management option.

With this view a study was conducted to identify the mealybugs infesting solanaceous and cucurbitaceous vegetables, its host range, natural enemies and associated ant species in Kerala. The study also aimed the molecular characterization of mealybugs that complement the morphological identification of species. An exploration of the tritrophic interaction in the brinjal, mealybug and its natural enemies was also carried out and the results are summarized below.

A total of six mealybug species *viz., Coccidohystrix insolita, Ferrisia virgata, Paracoccus marginatus, Phenacoccus solenopsis, Planococcus citri and Pseudococcus jackbeardsleyi* were recorded from the solanaceous and cucurbitaceous vegetables in Kerala. Besides, four mealybugs *viz., Crisicoccus hirsutus, Maconellicoccus hirsutus, Planococcus lilacinus* and *Rastrococcus iceryoides* infesting other vegetable crops were also recorded from Kerala. The mealybugs were identified and important characters were photographed.

The populations of *C. insolita* collected from different parts of Kerala exhibited a great deal of morphological variations and the molecular characterization studies revealed that all populations belongs to *C. insolita*. The intraspecific variations exhibited by the population may be environment induced.

A total of 113 plants under 73 genera belongs to 31 families were recorded as host plants of mealybugs infesting solanaceous and cucurbitaceous vegetables

in Kerala, of which 14 plants were observed as new host reports. The dominant families recorded as host plants of mealybugs were Asteraceae followed by Fabaceae, Malvaceae and Euphorbiaceae in Kerala. Among the plant categories, weeds recorded the highest mealybug infestation followed by vegetables and ornamentals. The cotton mealybug *P. solenopsis*, was recorded as the most dominant mealybug in Kerala, reported from 42 host plants belongs to 17 families.

Twenty species of predators belongs to five families under four orders *viz.* Coleoptera, Lepidoptera, Diptera and Neuroptera were recorded from different mealybugs in Kerala. The predominant family was Coccinellidae with 16 species under six genera and the majority belongs to the genus *Scymnus*. *Cacoxenus* sp. (Family: Drosophilidae) was recorded for the first time as a predator of *P. jackbeardsleyi*.

A study on parasitoids of mealybugs revealed the presence of 11 species belongs to 5 families, in which majority of the species recorded from the family Encyrtidae. Four new host-parasitoid associations were also recorded for the first time. The study also identified six hyperparasitoids under the family Encyrtidae, of which two species were recorded as new report which includes *Cheiloneurus* sp. and *Prochilonerus* sp. associated with the mealybug *P. solenopsis.*

A total of 14 species of ants belongs to nine genera under three subfamilies were found associated with mealybugs from the study area. The most dominant subfamily observed was Formicinae followed by Myrmicinae and Dolichoderinae. The present study revealed 15 new ant-mealybug associations for the first time.

The nucleotide sequences of 8 mealybugs were generated by molecular characterization. Comparison of the sequence with the NCBI database revealed the identity of the species that supports the morphological identification of mealybugs. A total of eight nucleotide sequences were submitted to NCBI GenBank and accession numbers were generated. The sequences were also

submitted to BOLD and illustrative barcodes were generated. The barcodes of *C. insolita*, *C. hirsutus* and *P. jackbeardsleyi* were generated for the first time in the world.

The study on gut endosymbionts of the mealybug, *C. insolita* was conducted for the first time. The study revealed a total of 15 phyla of endosymbionts in *C. insolita*, of which Proteobacteria was the predominant one followed by Euryarchaeota, Firmicutes, Bacteroidetes etc. The dominant class, order, family, genus and species of endosymbionts were recorded as Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae, *Pseudomonas* and *P. alcaligenes* respectively.

A field experiment was conducted at Instructional Farm, Vellayani to identify the tri-trophic interaction in brinjal, *C. insolita* and its natural enemies. Out of the ten brinjal cultivars, the lowest mean population of mealybugs was observed in Pusa Uttam which was statistically on par with Pusa Purple Long whereas the hybrid cultivar, Udit recorded the highest number of mealybugs which was statistically on par with Pink Long and Haritha.

The present study also recorded the lowest mean per cent leaf infestation in Pusa Purple Long which was statistically on par with Pusa Uttam whereas the hybrid cultivar, Udit was severely damaged by the mealybugs.

Ten brinjal cultivars were categorized into groups based on mean per cent leaf infestation caused by *C. insolita*. None of the cultivar was recorded to be immune to brinjal mealybug *C. insolita*. Among the tested cultivars, Pusa Purple Long was recorded under the group resistant whereas Pusa Uttam and Ponni were included in the group moderately resistant. The cultivars *viz.,* Pusa Kaushal, Pusa Shyamla and Neelima were contained in the group moderately susceptible while Pink Long and Green Long were designated under the susceptible category. The cultivars such as Udit and Haritha were included under the highly susceptible group based on the mean per cent leaf infestation caused by *C. insolita.*

The cultivar Haritha attracted the highest number of predators whereas the maximum mean population of spiders were observed in Pusa Purple Long.

The biophysical parameters of the host plant played a crucial role in determining the trophic level interactions in an ecosystem. Correlation and regression studies on the mean population of mealy bugs with biophysical parameters were carried out and the results showed that among the various biophysical parameters, trichome density, length width ratio of leaf and number of branches exhibited a significant positive correlation whereas the leaf thickness and plant height showed a non-significant negative correlation with mean population of mealybugs. The regression equation obtained was $Y = 0.099\ X_1 - 124.602\ X_2 - 57.811\ X_3 + 7.784\ X_4 - 0.856\ X_5 + 140.088$.

Correlation and regression studies on mean population of natural enemies with biophysical parameters were carried out and the results revealed that among the biophysical factors, plant height exhibited a significant negative correlation whereas leaf thickness and number of branches revealed a non-significant negative correlation with mean population of natural enemies. The trichome density and length width ratio of leaf showed a positive non-significant correlation with mean population of natural enemies. The regression equation obtained was $Y = 1.430\ X_2 - 4.099\ X_3 + 0.202\ X_4 - 0.076\ X_5 + 12.687$.

Biochemical traits of host plant plays a major role in conferring resistance to insect pests of crops and thus interfere with the population of natural enemies. Among the biochemical parameters of brinjal cultivars, total phenol content exhibited a significant negative correlation with the mean population of mealybugs whereas total chlorophyll content showed a significant positive correlation. The biochemical parameters such as total protein content and carotenoid content exhibited a non-significant negative correlation with the mean population of mealybugs whereas concentration of reducing sugars showed a non-significant positive correlation. The regression equation obtained was $Y = 2.636\ X_1 - 135.527\ X_2 - 21.739\ X_3 + 55.931\ X_4 - 235.137\ X_5 + 305.847$.

The correlation between biochemical parameters of brinjal cultivars with the mean population of natural enemies revealed that total phenol and carotenoid content exhibited a non-significant negative correlation whereas total protein content, reducing sugar and total chlorophyll content showed a non-significant positive correlation. The regression equation developed was $Y=-0.308\ X_1 +1.744\ X_2 -0.160\ X_3 + 0.892\ X_4 - 0.496\ X_5 + 0.013$.

The info-chemical mediated tri-trophic relationships among brinjal cultivars, mealybug and its natural enemies were studied by using multi-armed olfactometer and Y- shaped olfactometer assay. The preference of natural enemy, *C. zastrowi sillemi* towards the synomonal compounds extracted from the brinjal cultivars was evaluated in a multi-armed olfactometer and the results revealed that the cultivar Udit attracted the highest number of natural enemies while the cultivar Pusa Uttam attracted the lowest number. The variation in preference shown by the natural enemies may be related to the difference in volatile compounds emanating from the cultivar.

The GC-MS analysis of the synomonal compounds of the cultivar, Udit revealed a total of eleven compounds whereas the cultivar Pusa Uttam was recorded with a total of five compounds. The compounds *viz.,* diisooctyl phthalate, hexadecane, dodecane and heptadecane were common in the synomonal extracts of both cultivars, but the concentration of these compounds varied in two cultivars. The differences in compounds and their concentration in the volatile profile of the cultivars may lead to the highest preference of *C. zastrowii sillemi* to Udit and the lowest preference to Pusa Uttam.

Based on the results of multi-armed olfactometer, another experiment was devised to identify the relative response of *C. zastrowi sillemi* adults to the synomonal extracts of healthy and mealybug infested brinjal cultivar Udit (most preferred cultivar) in a Y- tube olfactometer. The results revealed that *C. zastrowi sillemi* adults showed more preference towards the synomonal compounds of mealybug infested plants than that of healthy synomonal extracts.

On comparing the volatile profile of synomones of mealybug infested and healthy plants revealed that the synomonal extracts of the mealybug infested cultivar Udit comprised of 11 compounds whereas healthy plant synomonal extracts contained nine compounds. The compounds *viz.,* hexadecane, heptadecane and dodecane were common in healthy and mealybug infested plant synomones. However, mealybug infested plant volatiles recorded a higher concentration than healthy plant synomones. The variation in the volatile profile of healthy and mealybug infested plants may lead to the differential response of natural enemies to the synomonal compounds.

The relative response of *C. zastrowi sillemi* towards the kairomonal compounds of mealybug *C. insolita* was evaluated in a Y tube olfactometer and the results revealed that the highest mean number of adult lacewings were attracted to the kairomonal compounds of mealybug compared to control.

The kairomonal extracts of the mealybug comprised of seven compounds, of which 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris (trimethylsiloxy) tetrasiloxane was recorded as the dominant compound. The composition of hydrocarbons in volatile blend may determine the selectivity of natural enemies towards the preferred host.

The result obtained from the study provides a basic idea on diversity of mealybugs, host range, natural enemies and associated ants in different agroecological regions of Kerala. The identification of various plant or herbivore derived compounds which can be used as cues or attractants for moderating the behaviour of natural enemies will be a breakthrough in integrated pest management. Enhancement of success of biological control options through manipulations in host plant- pest-natural enemy interactions coupled with exploitation of host plant resistance can result in the development of a sustainable pest management strategy.

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MEALYBUGS OF VEGETABLE ECOSYSTEMS AND TRITROPHIC INTERACTIONS OF BRINJAL MEALYBUGS

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ABSTRACT

The study on "Mealybugs of vegetable ecosystems and tritrophic interactions of brinjal mealybugs" was conducted at College of Agriculture, Vellayani during 2017 to 2020 with the objectives to identify mealybugs and their natural enemy fauna in solanaceous and cucurbitaceous vegetables, to carry out the molecular characterization of mealy bugs in solanaceous and cucurbitaceous vegetables and to find out the tritrophic interactions of mealybugs infesting brinjal.

An investigation on mealybug diversity in solanaceous and cucurbitaceous crops of Kerala revealed a total of six mealybug species *viz., Coccidohystrix insolita, Ferrisia virgata, Paracoccus marginatus, Phenacoccus solenopsis, Planococcus citri and Pseudococcus jackbeardsleyi.* Besides, four mealybugs *viz., Crisicoccus hirsutus, Maconellicoccus hirsutus, Planococcus lilacinus* and *Rastrococcus iceryoides* infesting other vegetable crops were also recorded from Kerala. The study also revealed that the population of mealybug, *C. insolita* collected from different regions of Kerala exhibited significant morphological variation. The molecular characterization studies proved that the population belongs to *C. insolita* and the variations may be environmental induced.

An exploration of the host range of mealybugs infesting solanaceous and cucurbitaceous vegetables in Kerala revealed a total of 113 plants under 73 genera belonging to 31 families, out of which 14 plants were recorded as new host reports. A rich natural enemy fauna on mealybugs belongs to five orders *viz.,* Coleoptera, Lepidoptera, Diptera, Neuroptera and Hymenoptera were documented from Kerala. Twenty species of mealybug predators from five different families were recorded, among which the predominant family was Coccinellidae with 16 species under six genera. Among the various predators collected, *Cacoxenus* sp. was recorded for the first time as a predator of *P. jackbeardsleyi*.

Eleven hymenopteran parasitoids belonging to five families were documented from mealybugs, of which the majority belongs to the family Encyrtidae. Four new host-parasitoid associations were also recorded for the first

time. The study also identified six hyperparasitoids under the family Encyrtidae, of which two species were recorded as new report which includes *Cheiloneurus* sp. and *Prochilonerus* sp. associated with the mealybug *P. solenopsis.*

The ants associated with mealybugs in different agroecosystems were also investigated and a total of 14 species of ants belonging to nine genera under three subfamilies were recorded, of which the most dominant subfamily was Formicinae followed by Myrmicinae and Dolichoderinae. The present study also revealed 15 new ant-mealybug associations for the first time.

The molecular characterization of eight mealybug species was carried out that complemented the morphological identification of species. A total of eight nucleotide sequences were submitted to NCBI GenBank and accession numbers were generated. The sequences were also submitted to BOLD and illustrative barcodes were generated. The barcodes of *C. insolita*, *C. hirsutus* and *P. jackbeardsleyi* were generated for the first time. The diversity of endosymbionts of the mealybug *C. insolita* was carried out for the first time. The study revealed a total of 15 phyla of endosymbionts on *C. insolita*, of which Proteobacteria was the predominant one.

An experiment was conducted at Instructional Farm, Vellayani to identify the tritrophic interaction in brinjal, *C. insolita* and its natural enemies. The study revealed that out of the ten brinjal cultivars evaluated, the lowest mean population of mealybugs was observed in Pusa Uttam whereas the hybrid cultivar, Udit recorded the highest number of mealybugs. The lowest mean per cent leaf infestation was recorded in Pusa Purple Long which was statistically on par with Pusa Uttam whereas the hybrid cultivar, Udit was severely damaged by the mealybugs. Among the tested cultivars, Pusa Purple Long was recorded under the group resistant whereas Udit and Haritha were included under the highly susceptible group based on the mean per cent leaf infestation caused by *C. insolita.* The cultivar Haritha attracted the highest number of predators whereas the maximum mean population of spiders were observed in Pusa Purple Long.

The correlation analysis on the mean population of mealy bugs with biophysical parameters revealed that trichome density, length width ratio of leaf and number of branches exhibited a significant positive correlation with mean population of mealybugs. Correlation studies on the mean population of natural enemies with biophysical parameters revealed that plant height exhibited a significant negative correlation whereas leaf thickness and number of branches revealed a non-significant negative correlation with the mean population of natural enemies. Among the biochemical parameters of brinjal cultivars, total phenol content exhibited a significant negative correlation with the mean population of mealybugs whereas total chlorophyll content showed a significant positive correlation. The correlation between biochemical parameters of brinjal cultivars with the mean population of natural enemies revealed that total phenol and carotenoid content exhibited a non-significant negative correlation whereas total protein content, reducing sugar and total chlorophyll content showed a non-significant positive correlation.

The info-chemical mediated interactions in brinjal cultivars, mealybug and its natural enemies were studied using a multi-armed olfactometer and Y-shaped olfactometer assay. The results revealed that the cultivar Udit attracted the highest number of natural enemy *Chrysoperla zastrowii sillemi* while the cultivar Pusa Uttam attracted the lowest number. The variation in preference shown by the natural enemies may be related to the difference in volatile compounds emanating from the host cultivar. The GC-MS analysis of the synomonal compounds of the cultivar, Udit revealed a total of eleven compounds whereas the cultivar Pusa Uttam was recorded with a total of five compounds. The Y shaped olfactometer studies revealed that *C. zastrowi sillemi* adults showed more preference towards the synomonal compounds of mealybug infested plants than that of healthy plant synomonal extracts. On comparing the volatile profile of synomones of mealybug infested and healthy plants revealed that the synomonal extracts of the mealybug infested cultivar Udit comprised of 11 compounds whereas healthy plant synomonal extracts contained nine compounds.

The relative response of *C. zastrowi sillemi* towards the kairomonal compounds of *C. insolita* was evaluated in a Y tube olfactometer and the results revealed that the highest mean number of adult lacewings were attracted to the kairomonal compounds of mealybug compared to control. The kairomonal extracts of the mealybug comprised of seven compounds and the composition of these hydrocarbons may determine the selectivity of natural enemies towards the preferred host.

The present study recorded a total of six mealybug species infesting solanaceous and cucurbitaceous vegetables and its host range, natural enemies and associated ants in different agro ecological regions of Kerala. The molecular characterization studies supported the morphological taxonomy and the sequences were submitted to NCBI Genbank and BOLD. The study also elucidated the interactions mediated by plant traits and info-chemicals in brinjal-mealybug-natural enemy tritrophic systems. The study recorded Pusa Purple Long as resistant cultivar and Udit and Haritha as highly susceptible cultivar to *C. insolita*. The plant traits *viz.,* trichome density, length width ratio of leaf, number of branches, total phenol content and total chlorophyll content exhibited significant correlation with mean population of mealybugs whereas plant height exhibited significant correlation with mean population of natural enemies. The study on info-chemical mediated tritrophic interactions revealed that the concentration and composition of volatile compounds determine the differential selectivity of natural enemies. The volatile compounds identified in this study can also be used as a cue in moderating the behavior of natural enemies in the ecosystems. So a thorough knowledge on the tritrophic relations in the ecosystem will aid in manipulating these interactions to devise a better pest management strategy.

സംഗ്രഹം

പച്ചക്കറി അധിഷ്ഠിത വിളകളിലെ മീലി മൂട്ടകളും വഴുതനയിലെ മീലിമൂട്ടയുടെ ത്രിതല പരസ്പര വ്യവഹാരങ്ങളും എന്ന വിഷയത്തിൽ വെള്ളായണി കാർഷിക കോളേജിൽ 2017-2020 കാലഘട്ടത്തിൽ നടത്തിയ പഠനത്തിന്റെ പ്രധാന ലക്ഷ്യങ്ങൾ വഴുതന വർഗ്ഗ വിളകളുടെയും മത്തൻ വർഗ്ഗ വിളകളുടെയും മീലിമൂട്ടകൾ, അവയെ ആക്രമിക്കുന്ന മിത്ര ഷഡ്പദങ്ങൾ എന്നിവയുടെ വൈവിധ്യം, മീലിമൂട്ടകളുടെ മോളികൂലർ പഠനം, വഴുതനയിലെ മീലിമൂട്ടയുടെ ത്രിതല പരസ്പര വ്യവഹാരങ്ങൾ എന്നിവയായിരുന്നു.

വഴുതന വർഗ്ഗ വിളകളെയും മത്തൻ വർഗ്ഗ വിളകളെയും ആക്രമിക്കുന്ന കോക്സിടോഹിസ്ട്രിസ്, ഫെർരിസിയ, പാരാകോക്കസ്, ഫെനകോക്കസ്, പ്ലാനോകോക്കസ്, സുഡോക്കോക്കസ് എന്നീ ആറ് മീലിമൂട്ടകളെയാണ് കേരളത്തിൽ നിന്നും കണ്ടെത്തിയത്. ഇവ കൂടാതെ മറ്റു പച്ചക്കറികളെ ആക്രമിക്കുന്ന നാല് മീലിമൂട്ടകളെ കൂടി ഈ പഠനം രേഖപ്പെടുത്തി. കേരളത്തിലെ പല പ്രദേശങ്ങളിൽ നിന്ന് ശേഖരിച്ച വഴുതനയിലെ മീലിമൂട്ടയായ കോക് സിടോഹിസ്ട്രിസ് ഇൻസോളിടാ അവയുടെ ബാഹ്യ പ്രകൃതിയിൽ വ്യത്യാസം പുലർത്തുന്നതായി കണ്ടെത്തി. എന്നാൽ മോളികൂലർ പഠനത്തിലൂടെ ഇവയെല്ലാം ഒരേ ജനുസ്സിൽ പെട്ടവയെന്നു മനസിലാക്കുകയും അവയുടെ വ്യത്യാസം പരിസ്ഥിതിയിലും കാലാവസ്ഥയിലും ഉള്ള വ്യതിയാനങ്ങൾ കൊണ്ടന്നും ഈ പഠനം കണ്ടെത്തി.

പ്രസ്തുത പഠനം രേഖപ്പെടുത്തിയ മീലിമൂട്ടകൾ കേരളത്തിൽ 113 സസ്യങ്ങളെ അക്രമിക്കുന്നതായി കണ്ടെത്തുകയും അവയിൽ തന്നെ 14 സസ്യങ്ങളിൽ ഇവയുടെ ആക്രമണം ആദ്യമായി രേഖപ്പെടുത്തുകയും ചെയ്തു. മീലിമൂട്ടകളെ ആക്രമിക്കുന്ന മിത്ര ഷഡ്പദങ്ങളുടെ വൈവിധ്യം പഠിച്ചതിൽ നിന്നും അവ കോളിയോപ്റ്റെറ, ലെപിഡോപ്റ്റെറ, ഡിപ്റ്റെറ, ഹൈമേനോപ്റ്റെറ എന്നീ വിഭാഗങ്ങളിൽ ഉൾപെടുന്നവയെന്നു മനസിലാക്കി. കോക്സിനെല്ലിടെ കുടുംബത്തിലെ പെട്ട സുന്ദരി വണ്ടുകളാണ് മിത്രഷഡ്പദങ്ങളിൽ പ്രധാനിയായി രേഖപ്പെടുത്തിയത്. ഇവ കൂടാതെ ഹൈമേനോപ്റ്റെറ വിഭാഗത്തിൽപെട്ട 11 മിത്ര പരാദങ്ങളെയും 6 ഹൈപ്പർ പരാദങ്ങളെയും പഠനം രേഖപെടുത്തുകെയും ചെയ്തു. മീലിമൂട്ടകളുടെ സഹകാരിയായ 15 ഇനത്തിലെ പെട്ട ഫോർമിസിനെ, മിർമിസിനെ, ഡോളിക്കോടെറിനെ എന്നീ വിഭാഗങ്ങളിൽ ഉള്പെട്ട ഉറുമ്പുകളെ കണ്ടെത്തുകയും ചെയ്തു.

മീലിമൂട്ടകളുടെ മോളികൂലർ പഠനങ്ങൾ അവയുടെ ടാക്സോനാമിക് പഠനങ്ങളെ സാധുകരിക്കുന്നതായിരുന്നു. എട്ടു മീലി മൂട്ടകളുടെ ന്യൂക്ളിയോടൈഡ് സികവൻസുകൾ ലഭിക്കുകയും ജീൻ ബാങ്കിൽ അവ നിക്ഷേപിച്ചു അക്സെഷൻ

നമ്പറുകൾ ലഭിക്കുകയും ചെയ്തു . കൂടാതെ ബാർകോഡ് ഓഫ് ലൈഫ് ഡാറ്റബേസിൽ ഇവയുടെ ബാർകോഡുകൾ സമർപ്പിക്കുകയും ചെയ്തു. ഇതിൽ മൂന്ന് ബാർകോഡുകൾ ലോകത്തിൽ തന്നെ ആദ്യമായിട്ടാണ് പ്രസ്തുത ഡാറ്റബേസിൽ സമർപ്പിക്കുന്നത്. കോക്സിടോഹിസ്ട്രിസ് ഇൻസോളിടാ എൻഡോസിമ്പിയൻറ്കളെപ്പറ്റിയുള്ള പഠനം ലോകത്തിൽ ആദ്യമായി നടത്തുകയും 15 വിഭാഗത്തിൽ പെട്ട സൂക്ഷ്മ ജീവികളെ കണ്ടെത്തുകയും അവയിൽ പ്രോട്ടിയോബാക്ടീരിയ എന്ന വിഭാഗം ആണ് പ്രധാനി എന്ന് രേഖപ്പെടുത്തുകയും ചെയ്തു.

വഴുതന വിളയും കോക്സിടോഹിസ്ട്രിസ് ഇൻസോളിടാ മീലി മൂട്ടയും അവയുടെ മിത്ര ഷഡ്പദങ്ങളും തമ്മിലുള്ള ത്രിതല പരസ്പര വ്യവഹാരങ്ങൾ പഠിക്കുന്നതിനായി നടത്തിയ പഠനത്തിൽ നിന്ന് ഏറ്റവും കുറവ് മീലി മൂട്ടകൾ കാണപ്പെടുന്നത് പുസ ഉത്തം എന്ന ഇനത്തിലാണെന്നും ഏറ്റവും കൂടുതൽ കാണപ്പെടുന്നത് ഹൈബ്രിഡ് ഇനമായ ഉദിത് ആണെന്നും കണ്ടെത്തി. കൂടാതെ മീലി മൂട്ടകളുടെ രൂക്ഷമായ ആക്രമണ ലക്ഷണങ്ങൾ കാണപെടുന്നത് ഉദിത് ഇനത്തിലും കുറവ് കാണപ്പെടുന്നത് പുസ പർപ്പിൾ ലോങ്ങ്, പുസ ഉത്തം എന്ന ഇനങ്ങളിലും ആണെന്ന് കണ്ടെത്തി. ഏറ്റവും കൂടുതൽ മിത്ര ഷഡ്പദങ്ങളെ ഹരിത എന്ന ഇനത്തിലും ഏറ്റവും കൂടുതൽ ചിലന്തികളെ പുസ പർപ്പിൾ ലോങ്ങ് എന്ന ഇനത്തിലും രേഖപ്പെടുത്തി.

മീലിമൂട്ടകളുടെയും മിത്ര ഷഡ്പദങ്ങളുടെയും എണ്ണവും ബയോ ഫിസിക്കൽ പരാമീറ്റർസും തമ്മിലുള്ള ബന്ധം മനസിലാക്കൻ വേണ്ടി നടത്തിയ പഠനത്തിൽ നിന്നും ട്രൈകോം ഡെന്സിറ്റി, ലെങ്ത് - വിഡ്ത് അനുപാതം, ശിഖരങ്ങളുടെ എണ്ണം എന്നിവ മീലി മൂട്ടകളുടെ എണ്ണവുമായി പോസിറ്റീവ് കോറീലേഷനും ചെടിയുടെ ഉയരം മിത്രഷഡ്പദങ്ങളുടെ എണ്ണവുമായി നെഗറ്റീവ്കോറീലേഷനും കാണിക്കുന്നതായി കണ്ടെത്തി.

ഇൻഫോ - കെമിക്കൽ വഴിയുള്ള ത്രിതല വ്യവഹാരങ്ങൾ പഠിക്കുന്നതിനായി നടത്തിയ മൾട്ടി ആറ്മഡ് ഓൾഫാക്ടോമീറ്റർ പരീക്ഷണങ്ങളിൽ നിന്നും ഉദിത് എന്ന ഇനത്തിന്റെ സിനമോണൽ സത്തിനോടു ക്രയ്സോപെർള ഇനത്തിലെ പെട്ട മിത്ര ഷഡ്പദങ്ങൾ കൂടുതൽ ആകർഷണം കാണിക്കുകയും എന്നാൽ പുസ ഉത്തം എന്ന ഇനത്തോട് ഏറ്റവും കുറവ് ആകർഷണം കാണിക്കുകയും ചെയ്തു. ജി സി- എം എസ് അനാലിസിസ് നടത്തിയതിൽ നിന്നും ഉദിത് ഇനത്തിന്റെ സിനമോണൽ സത്തിൽ 11 ഘടകങ്ങൾ കണ്ടെത്തുകയും പുസ ഉത്തം സിനമോണൽ സത്തിൽ നിന്നും 5 ഘടകങ്ങൾ കണ്ടെത്തുകയും ചെയ്തു.

വൈ-ഓൾഫാക്ടോമീറ്റർ പഠനങ്ങൾ വഴി മീലിമൂട്ട ആക്രമണമേറ്റ ഉദിത് ഇനത്തിന്റെ സിനമോണൽ സത്തിനോട് ആക്രമണമേൽകാത്ത സിനമോണൽ സത്തിനേക്കാൾ ആകർഷണം ക്രയ്സോപെർള കാണിക്കുന്നതുമായി കണ്ടെത്തി.

ജി സി- എം എസ് അനാലിസിസ് നിന്നും മീലിമൂട്ട ആക്രമണമേറ്റ ഉദിത് ഇനത്തിന്റെ സിനമോണൽ സത്തിൽ 11 ഘടകങ്ങളും ആക്രമണമേൽകാത്ത സിനമോണൽ സത്തിൽ 9 ഘടകങ്ങളും കണ്ടെത്തി. ഇതിൽ നിന്നും സിനമോണൽ സത്തിൽ അടങ്ങിയ ഘടകങ്ങളുടെ എണ്ണവും അവയുടെ അളവും മിത്രഷഡ്പദങ്ങളുടെ ആകർഷണത്തെ തീരുമാനിക്കുന്നു എന്ന് അനുമാനിക്കാം.

വൈ -ഓൾഫാക്ടോമീറ്റർ ഉപയോഗിച്ച് മീലിമൂട്ടകളുടെ കയറോമോൺസ്നോടുള്ള മിത്രഷഡ്പദങ്ങളുടെ ആകർഷണം പഠിച്ചതിൽ നിന്നും കയറോമോൺ, ക്രയ്സോപെർള ഇനത്തിലെ പെട്ട മിത്ര ഷഡ്പദങ്ങളെ കൂടുതലായി ആകർഷിക്കുന്നതായി കണ്ടെത്തി. ജി സി- എം എസ് അനാലിസിസ് വഴി കയറോമോൺന്റെ 7 ഘടകങ്ങളെ രേഖപെടുത്തുകയും ഘടകങ്ങളുടെ എണ്ണവും അവയുടെ അളവും മിത്രഷഡ്പദങ്ങളുടെ ആകർഷണത്തെ തീരുമാനിക്കുന്നു എന്നും കണ്ടെത്തി.

പ്രസ്തുത പഠനം വഴുതന വർഗ്ഗ വിളകളെയും മത്തൻ വർഗ്ഗ വിളകളെയും ആക്രമിക്കുന്ന 6 മീലിമൂട്ടകളെയും മറ്റു പച്ചക്കറികളെ ആക്രമിക്കുന്ന നാല് മീലിമൂട്ടകളെയും അവയുടെ ആക്രമണമേറ്റ സസ്യങ്ങൾ, മിത്ര ഷഡ്പദങ്ങൾ, സഹകാരിയായി വർത്തിക്കുന്ന ഉറുമ്പുകൾ എന്നിവയുടെ വൈവിധ്യം കേരളത്തിൽ നിന്നും രേഖപ്പെടുത്തി. മീലിമൂട്ടകളുടെ ടാക്സോനാമിക് പഠനങ്ങളെ മോളികൂലർ പഠനങ്ങൾ സാധുകരിക്കുകയും മീലി മൂട്ടകളുടെ ന്യൂക്ളിയോടൈഡ് സികവൻസുകൾ ജീൻ ബാങ്കിലും ബോൾഡ് ഡാറ്റാബേസിലും സമർപ്പിക്കുകയും ചെയ്തു. വഴുതന വിളയും കോക്സിടോഹിസ്ട്രിസ് ഇൻസോളിടാ മീലി മൂട്ടയും അവയുടെ മിത്ര ഷഡ്പദങ്ങളും തമ്മിലുള്ള ത്രിതല പരസ്പര വ്യവഹാരങ്ങൾ പഠിക്കുകയും ഇനങ്ങളുടെ ബയോ ഫിസ് സിക്കൽ, ബയോ കെമിക്കൽ പരാമീറ്ററുകളും മീലിമൂട്ടയുടെയും മിത്ര ഷഡ്പദങ്ങളുടെയും എണ്ണവുമായിട്ടുള്ള കോറിലേഷനുകൾ കണ്ടെത്തുകയും ചെയ്തു. ഇൻഫോ - കെമിക്കൽ വഴിയുള്ള ത്രിതല വ്യവഹാരങ്ങൾ പഠിച്ചതിൽ നിന്നും വഴുതന ഇനങ്ങളിൽ അടങ്ങിയിട്ടുള്ള സിനമോണൽ, മീലിമൂട്ടകളുടെ കയറോമോൺ ഘടകങ്ങൾ എന്നിവയുടെ എണ്ണവും അവയുടെ അളവും മിത്രഷഡ്പദങ്ങളുടെ ആകർഷണത്തെ തീരുമാനിക്കുന്നു എന്നും കണ്ടെത്തി. ത്രിതല വ്യവഹാരങ്ങളെ പറ്റിയുള്ള പഠനം സന്തുലിതമായ ഒരു കീട നിയന്ത്രണ മാർഗ്ഗം ഉണ്ടാകുന്നതിനായി സഹായിക്കുന്നു.

Appendices

Appendix 1. Species distribution pattern of mealybug species in Kerala

Sl. No	Mealybug species	Location	GPS
1	*Coccidohystrix insolita*	COA, Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”
		Balaramapuram	N $8^0$25’14.01” E 77^0 2’25.68
		CPCRI, Kayamkulam	N $9^0$8’51.02” E 76^0 30’50.82”
		COH, Vellanikkara	N 10^0 32’43.5” E 76^0 17’0.4”
		RARS Pilicode	N 12°12'09.7" E 75°09'53.4"
2	*Phenacoccus* sp.	COA, Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”
		Balaramapuram	N $8^0$25’14.01” E 77^0 2’25.68
		Thiruvallom	N $8^0$24’14.87” E 76^0 59’28.23”
		Panagode	N $8^0$25’22.62” E 76^0 58’ 17.4252”
		FSRS, Sadanandapuram	N 8°58'53.9" E 76°48'39.5"
		Ummannoor	N 8^0 56’7.34568” E76^0 48’ 31.83084”
		Paravoor	N 8°48'52.6" E 76°40'11.3"
		Perumkulam	N 9°02'32.4" E 76°45'16.9"
		Enathu	N 9°05'28.4" E 76°45'16.3"
		Koickal chantha	N 9°11'5" E 76°33'20.7"
		ORARS, Onattukkara	N $9^0$10’33.46” E$76^0$30’59.41”
		RARS, Kumarakom	N $9^0$37’39.64” E 76^0 25’53.2”
		KVK, Ernakulam	N 10°02'33.5"

			E 76°12'24.9"
		COH, Vellanikkara	N 10^0 32’43.5” E 76^0 17’0.4”
		KVK, Thrissur	N 10°32'49.3" E 76°16'05.5"
		RARS, Pattambi	N $10^0$48’40.12812” E 76^0 11’ 25.82916”
		KVK, Tavanur	N $10^0$51’12.36348” E $75^0$59’ 13.15032”
		PRS, Panniyur	N 12^0 4’ 47.6202” E 75^0 23’41.84016”
		COA, Padannakkad	N 12°11'41.9" E 75°11'17.4"
3	*Ferrisia virgata*	COA, Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”
		Karavaram	N 8°45'09.4" E 76°48'52.9"
		Chenkal	N 8°22'23.2" E 77°06'02.1"
		Poovattor	N 9°03'22.9" E 76°45'02.0"
		Kadakkal	N 8°49'48.5" E 76°55'12.2"
		Ezhamkulam	N 9°09'10.4" E 76°46'17.6"
		Prakkanam	N 9°16'14.88684" E 76°44'30.62004"
		ORARS, Onattukkara	N $9^0$10’33.46” E$76^0$30’59.41”
		RARS, Kumarakom	N $9^0$37’39.64” E 76^0 25’53.2”
		CRS, Pampadumpara	N 9°47'56.0" E 77°09'41.5"
		Prakandam	N 9°47'51.79092" E 77° 8'59.36748"
		Valiyathovala	N 9°48'8.45028" E 77° 7'57.04824"
		Mannakkudi	N 9°47'31"

			E 77° 8'1.58"
		Anchumukku	N 9°48'10.53504" E 77° 7'35.46552"
		Munnar	N 10°05'25.7" E 77°03'15.9"
		COH, Vellanikkara	N 10^0 32’43.5" E 76^0 17’0.4"
		KVK, Thrissur	N 10°32'49.3" E 76°16'05.5"
		Muthalamada	N 10°38'14.3" E 76°48'02.4"
		Vattamkulam	N 10°47'24.6" E 76°01'54.2"
		Kavilumpara	N 11°42'13.2" E 75°47'16.1"
		Manjappara	N 11°36'14.06196" E 76°12'35.45856"
		Aandoor	N 11°35'17.16828" E 76°1'32.21832"
		RARS, Pilicode	N 12°12'09.7" E 75°09'53.4"
4	*Paracoccus marginatus*	COA, Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”
		Ummannoor	N 8°56'02.6" E 76°48'45.1"
		ORARS, Onattukkara	N $9^0$10’33.46” E$76^0$30’59.41”
		COH, Vellanikkara	N 10^0 32’43.5” E 76^0 17’0.4”
		RARS, Ambalawayal	N 11°36'59.8" E 76°12'52.2"
		RARS, Pilicode	N 12°12'09.7" E 75°09'53.4"
5	*Maconellicoccus hirsutus*	Koickal chantha	N $9^0$11’5” E 76^0 33’20.7”
		RARS, Kumarakom	N $9^0$37’39.64” E 76^0 25’53.2”
6	*Planococcus lilacinus*	COA, Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”

7	*Planococcus citri*	COA, Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”
		Karavaram	N 8°45'09.4" E 76°48'52.9"
		FSRS, Sadanandapuram	N 8°58'53.9" E 76°48'39.5"
		CPCRI, Kayamkulam	N $9^0$8’51.02” E 76^0 30’50.82”
		RARS, Ambalawayal	N 11°36'59.8" E 76°12'52.2"
		PRS, Panniyur	N 12^0 4’ 47.6202” E 75^0 23’41.84016”
8	*Pseudococcus jackbeardsleyi*	COA, Vellayani	N $8^0$25’46.6788” E 76^0 59’15.02016”
		Karavaram	N 8°45'09.4" E 76°48'52.9"
		Koickal chantha	N $9^0$11’5” E 76^0 33’20.7”
		RARS, Ambalawayal	N 11°36'59.8" E 76°12'52.2"
9	*Crisicoccus hirsutus*	Kulathoor	N 8°32'19.8" E 76°53'07.6"
10	*Rastrococcus iceryoides*	Koickal chantha	N $9^0$11’5” E 76^0 33’20.7”
		Muthalamada	N 10°38'14.3" E 76°48'02.4"