DIVERSITY OF ROOT MEALYBUGS OF KERALA

By

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(2018-11-058)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

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KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"DIVERSITY OF ROOT MEALYBUGS OF KERALA"** is a bona fide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar titles, of any other University or Society.

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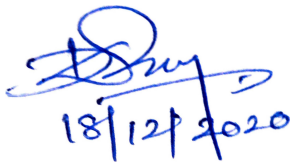
Certified that this thesis entitled **"DIVERSITY OF ROOT MEALYBUGS OF KERALA"** is a record of research work done independently by **Mr. Sachin G. Pai (2018-11-058),** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.



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DEDICATED TO MY PARENTS

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INTRODUCTION

INTRODUCTION

Mealybugs (Homoptera: Pseudococcidae) are oval, soft-bodied insects that are named by the appearance of white waxy powder coating on the surface of their body. They often congregate in large numbers and feed extensively on the phloem sap of their host plants. These are one of the most important sucking pests of crop plants which feed on herbaceous as well as woody plants. They cause damage to the plants not only by sucking the sap but also as vectors of viral diseases of crop plants. Mealybugs suck phloem sap and the excess water is excreted as a sugary substance called honeydew which encourages the black growth of a *Capnodium* fungus. The honeydew also serves as nutritive food for ants associated with these mealybugs. These ants in turn will give protection to the mealybugs from their natural enemies and also helps in dispersal to newer areas.

The mealybugs infesting roots of plants are termed as subterranean mealybugs or root mealybugs. They suck sap from the main root, lateral roots as well as from the root hairs. Continuous desapping result in loss of vigour, yellowing, wilting and finally death of infested plants. Root mealybugs have been reported from many of the economically important crops like black pepper, cardamom, pineapple, banana, coffee *etc*. Due to their cryptic nature and subterranean habitat, proper diagnosis in the early stage of infestation is very difficult leading to huge economic losses. Apart from that, false identification of mealybugs creates a challenge to management by reducing the efficiency of crop protection measures and results in the injudicious application of pesticides. Limited reliable surveys and extreme difficulty in proper identification and characterizations of mealybug species worsen the case. In reality, taxonomic identification of mealybugs is mainly based on adult female characters which demand specialized taxonomic knowledge and skill. Moreover, it is time consuming and cumbersome process. Variation in the environmental conditions may induce changes in the phenotypes, making it extremely difficult to differentiate between morphologically similar cryptic species (Cox, 1983; Charles *et al*., 2000). Conventional taxonomic identification is hard when specimens are of immature stages.

These difficulties can be overcome by complementing morphological and molecular characterization to identify the species. In fact, after taxonomic identification of a reference specimen by morphological examination and characterized by DNA sequencing, a distinct barcode can be generated and after that any new sample having the same DNA sequence can be identified quickly. This will also help to avoid repetitive identification of the most common species by the taxonomist. Both morphological identification and molecular characterization should go hand in hand. In the recent past, the root mealybug infestation from various crop plants from several areas of Kerala has been noticed. Most of the root mealybugs are of quarantine importance and strict quarantine regulations are to be undertaken to prevent the spread of pest infestation to other areas. Moreover, these mealybugs are often association with ants for protection and distribution to new areas and new host plants. This symbiotic association can be obligate or facultative and thus can pose a serious threat to the crop plants. However, there are limited studies on root mealybugs, their host range, seasonal incidence and association with ants so far.

Documentation and identification of the root mealybugs and associated ant species, the study of the seasonal incidence of the mealybug population with soil and weather parameters are important to develop an efficient management strategy against root mealybugs. Hence the present study entitled ‘Diversity of root mealybugs of Kerala’ was undertaken with the following objectives :

- Identification of root mealybugs and associated ant species
- To study the seasonal incidence, host range and geographical distribution of major root mealybugs

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Root mealybugs are group of pseudococcids that feed on the underground plant parts, and they are well adapted to subterranean habitat. They cause serious damage to a cultivation of a number of agricultural and horticultural crops. The subterranean nature of these mealybugs makes it very difficult to detect in the field in advance which in turn can cause their population to buildup leading to economic losses to the farmer. The relevant literature on root mealybug pests of horticultural crops are reviewed here.

2.1 ROOT MEALYBUG PEST OF HORTICULTURAL CROPS

Mealybugs are one of the most important pests of horticultural crops and have been known to cause widespread damage both directly and indirectly as vectors of diseases. Species of mealybugs which feed exclusively on the roots are emerging as serious pests of many horticultural crops.

2.1.1 Fruit crops

The attack of root mealybugs on various fruit crops were reported by many workers from different regions of the world. Puttarudriah and Eswaramurthy (1976) reported *Planococcoides* sp. nr. *robustus* attacking roots of grapes, mango and weed *Coniza ambigua* DC. from Kolar district of Karnataka. *Dysmicoccus brevipes* Cockerell was reported to infest the roots of *Musa* sp. L. (Butani, 1979). It was also reported as a major pest of pineapple and commonly called pineapple pink mealybug (Beardsley, 1993; Gonzalez – Hernandez *et al*., 1999). The root mealybug *Rhizoecus kondonis* Kuwana was reported to be feeding on the roots of *Prunus domestica* L. and alfalfa in California by Godfrey and Pickel (1998). Williams and Matile-Ferrero (1999) reported ten species of mealybugs infesting the members of the genus *Musa* and *Ensete* and among these, the mealybug, *Cataenococcus ensete* Williams and Matile-Ferrero was identified as the major pest of enset in Ethiopia.

The infestation of *Geococcus coffeae* Green was noticed on the roots of citrus, mango, pineapple by Hara *et al*. (2001) in Hawaii. A major mealybug infesting banana and pineapple in Taiwan was reported as be *D. brevipes* (Huang *et al*., 2002). The vine mealybug, *Planococcus ficus* Signoret was found to be feeding both on foliage and roots of grape vine (Walton and Pringle, 2004).

Smitha *et al*. (2005) reported two species of mealybugs *viz*., *G. coffeae* and *G. citrinus* Kuwana infesting banana roots from Kerala. Mukhopadhyay *et al*. (2010) reported the occurrence of *Paraputo* sp. infesting the roots of mulberry plants from high altitude areas like Kalimpong and Darjeeling of West Bengal. The presence of root mealybug, *Xenococcus annandalei* Silvestri on the roots of grapes, banana, mango, Jack tree and wild jack tree (*Artocarpus hirsutus* Lam.) were reported from Bangalore and Idukki districts of south India (Rajagopal *et al*., 1997; Deepthy *et al.,* 2017). Pai *et al.* (2020) reported *Formicococcus polysperes* Williams infesting the roots of Avocado (*Persea americana* Mill.) seedling from Wayanad district of Kerala for the first time.

2.1.2 Plantation and spice crops

The infestation of coconut roots by the mealybug, *Rhizoecus cocois* Williams was observed by Nair *et al*. (1980) from Kazhakkoottam in Kerala. Coffee root mealybug*, G. citrinus* was known to infest the roots of betel vine (*Piper betle* L.) from North Arcot district of Tamil Nadu and Maharashtra (Muthukrishnan *et al.,* 1958; Williams, 1985).

Williams (2004) reported *F. polysperes* on the roots of black pepper (*Piper nigrum* L.) and betel vine from Kerala and Maharashtra, respectively. Studies by Devasahayam *et al.* (2009) in the southern states of Kerala and Karnataka revealed five species of root mealybugs infesting black pepper *viz., Planococcus lilacinus* Cockerell*, P. citri* Risso*, Planococcus* sp., *Ferrisia virgata* Cockerell and *D. brevipes* .

Josephrajkumar *et al*. (2012) observed the presence of buff coconut mealybug, *Nipaecoccus nipae* Maskell on the roots of coconut seedlings for the first time in Kayamkulam, Kerala. Severe infestation of *F. polysperes* on the rhizomes of ginger and

turmeric from the states of Meghalaya and Kerala was reported by Firake *et al.* (2015); Ummer *et al*. (2015) and Firake *et al.* (2018).

The hypogeal mealybug, *X. annandalei* was found to be infesting the roots of coconut palms and *Ficus obtusa* Hassk. in the Barakuda islands of Orissa for the first time by Silvestri (1924) and later from Mysore (Williams, 1978). The pest was also reported from Kerala during 2014 infesting the succulent tender roots of major crops like cardamom, cocoa, black pepper, tea, coffee, nutmeg, turmeric, and ginger in addition it was also seen infesting many weed species *viz.,* coat buttons (*Tridax procumbens* L.), hen's nettle *(Laportea interrupta* L.), black night shade (*Solanum nigrum* L.) and ficus (*F. obtusa*) (Deepthy *et al*., 2017)

Two new species mealybugs were described from Africa infesting coffee roots *viz., Planococcus fungicola* Watson and Cox from Kenya and *Planococcus radicum* Watson and Cox from Nigeria and Tanzania (Watson and Cox, 1990).

2.1.3 Vegetable crops and tuber crops

Gupta and Norman (1975) observed that tomato cultivars drastically wilted and died within 30 days of transplanting, which was attributed to high infestation and feeding by *D. brevipes* on the roots. Williams (1985) observed the presence of *G. coffeae* on the roots of sweet potato from Tamil Nadu, India. The presence of *D. brevipes* was reported on the exposed roots of brinjal (Hara *et al*., 2001). Nedunchezhiyan *et al*. (2011) observed the presence of *Rhizoecus amorphophalli* Betrem on the corms of elephant foot yam (*Amorphophallus paeoniifolius* Dennst.) both in field and storage conditions. Halder *et al*. (2020) recorded *F. polysperes* on the roots of a new host taro (*Colocasia esculenta* L.) from the Indian state of Nagaland, which caused damage both in field and storage condition.

2.1.4 Ornamental plants

Hara *et al*. (2001) reported the presence of *D. brevipes* on the roots of caladium, canna and palms and also presence of *G. coffeae* and *Rhizoecus hibisci* Kawai and Takagi on the roots of the following plants *viz*., croton, aglonema, dieffenbachia, cyperus, ferns,

oleander, palms, philodendron, schefflera, syngonium, calathea, *Serrisa* spp., and 'Tifdwarf' bermudagrass.

Sridhar *et al*. (2012) observed the lantana mealybug *Phenacoccus parvus* Morrison infesting the roots and the collar region of China aster (*Callistephus chinensis* L.) from Bangalore, India. Soo-Jung *et al*. (2013) recorded the presence of *Ripersiella multiporifera* Jansen and *Rhizoecus albidus* Goux on the roots of imported dracaena plants (Dracaenaceae) and *Schlumbergera truncata* (Haw.) Moran (Cactaceae), growing in the greenhouses of Korea.

2.1.5 Other crops

Green gram (*Vigna radiata* L.) was reported as a new host for the mealybug *G. coffeae* from Tamil Nadu by Rao *et al*. (1974). Rajagopal *et al*. (1982) observed *D. brevipes* infesting the root nodules of legumes, groundnut (*Arachis hypogea* L.) and red gram (*Cajanus cajan* L.) for the first time in south India. The infestation of *D. brevipes* was observed on both aerial and root nodules of groundnut (Singh *et al*., 1986).

Pineapple root mealybug, *D. brevipes* was reported to be feeding on the exposed roots of sugarcane (Beardsley, 1982 as cited by Beardsley, 1993 and Hara *et al.,* 2001).

Williams (1996) reported that *Rhizoecus carolinensis* Beardsley, *R. saintpauliae* Williams, *R. hibisci* Kawai and Takagi and *R. bacorum* Williams were polyphagous and potential pests of greenhouse and ornamental potted plants of quarantine significance. Malumphy (2012) recorded the Asian root mealybug, *Ripersiella planetica* (Coccoidea, Rhizoecidae) in Europe. *Paraputo lingnani* Ferris, later renamed as *Formicococcus lingnani* Ferris by Joshi *et al.* (2020) was reported to be feeding on the roots of the weed, *Cyperus rotundus* L. from mixed plantations of Wayanad district (Pai *et al.,* 2019).

2.2 Ants associated with root mealybug

The mutualistic relationship of ants with large number of aphids and mealybugs is known from time immemorial. These insects produce honeydew which is a nutritious source of food for ants and in turn the ants provide protection to these insects from natural

enemies. This relationship between ants and homopteran in which both organisms are benefited and improving their lives can be called trophobiosis (Way 1963; Gullan 1997; Flatt and Weisser, 2000; Kondo and Gullan, 2004).One of the most important reasons for the evolutionary success of hemipteran insects and hymenopteran insects is trophobiosis.

The queens of *Tetraponera* sp. of ants were found to be carrying and associated with unidentified mealybugs of *Chaetococcus* sp. in Malay peninsula (Klein *et al*., 1992). Three species of *Pseudolasius* were found to have trophobiotic relationship with eight species of scale insects, and association beyond pure trophobiosis was observed in five species *viz*., *Planococcoides* sp., *Maconellicoccus multipori* Takahashi and three species of *Rhizoecus* (Malsch *et al*., 2001). LaPolla *et al*. (2002) reported that the ant *Acropyga epedena* Snelling was having obligate association with coccids and both males and queens of these ants obtain their energy source from the mealybugs they tend, and the female transports the trophobiont during her mating flight. Smith *et al*. (2007) reported that the ant *A. epedana* was mutually associated with root mealybug *Rhizoecus colombiensis.* The ants during their reproductive flight carried the females of the mealybugs between the mandibles, which indicated that there is a vertical transfer of mealybugs with their ant hosts. Schneider and LaPolla (2011) observed that the mealybug tribe Xenococcini (Hemiptera: Pseudococcidae) consisting of three genera *Xenococcus* Silvestri, *Eumyrmococcus* Silvestri and *Neochavesia* Williams & Granara de Willink were found to be having trophobiotic association with ants belonging to the genus *Acropyga* Roger (Hymenoptera: Formicidae). *Ishigakicoccus shimadai* Tanaka*,* a newly reported species from Japan was having trophobiotic association with ant, *Acropyga yaeyamensis* Terayama & Hashimoto (Tanaka, 2016).

The blueberry mealybug, *Dysmicoccus vaccinii* Miller was found to feed on the roots of blueberry plants and was associated with two ant species, *Acanthomyops claviger* (Roger) and *Lasius neoniger* Emery (Stuart and Polavarapu, 2002). Jahn *et al*. (2003) stated that the most common ant species associated with pineapple mealybugs were *Solenopsis* and *Pheidole* species. In Southeast United States, the invasive ant species *Solenopsis*

invicta Buren depends upon mealybugs feeding on grasses to derive their carbohydrate source. The major species of mealybug associated was the invasive *Antonina graminis* Maskell. It was also observed that population of mealybugs increased seriously with its proximity to the ant mounds (Helms and Vinson, 2003). Enset root mealybug was known to be associated with unidentified species of ants which facilitates its distribution in field and protects it from natural enemies (Addis, 2005; Azerefegne *et al*., 2009). According to Devasahayam *et al*. (2009), five species of ants *viz., Technomyrmex* sp., *Anaplolepis* sp., *Crematogaster* sp., and two unidentified ant species were associated with root mealybug colonies in black pepper. Deepthy *et al*. (2017) observed that ant, *Acropyga acutiventris* Roger associated with the mealybug *X. annandalei* helped in its spread to different host plants.

2.3 DNA barcoding for species determination

Identification of species is important both in terms of understanding biodiversity and also in terms of identifying a pest. An accurate and rapid method of identification of a pest is one of the most an important step in the pest management programme. Expertise in taxonomy is essential for identification of an insect and is time consuming and complex process. To overcome these problems molecular characterization by DNA barcoding can be undertaken for rapid and reliable method of species identification.

Discrimination of organisms based on short sequences of DNA was suggested by Nanney (1982). Folmer *et al*. (1994) reported that mitochondrial cytochrome oxidase one (mtCO1) can be used in identification of eleven invertebrate phyla using universal primers for PCR reaction, and he concluded that the sequences generated were of good quality for phylogenetic analysis. Hebert *et al*. (2003) proposed a technique for accurate identification of biological specimens by designing a set of primers for amplifying 648 bp region of mtCO1 and entitled it as DNA barcoding and suggested that identification using mtCO1 system will become the reliable, cost effective and accessible solution to the problems in species identification.

2.3.1 DNA barcoding of mealybugs

Gullan *et al*. (2003) utilized region two of mtCO1 for reporting a new pest species belonging to genus *Ferrisia* Fullaway from United States. Pieterse *et al*. (2010) developed a technique for easy and rapid identification of immature stages of mealybugs on horticultural consignments for export using 749 bp region of the mtCO1 gene.

Malausa *et al*. (2009) employed five DNA markers to distinguish the species belonging to cryptic taxa of mealybugs. Ashfaq *et al*. (2010) were the first to undertake DNA based characterization of cotton mealybugs of Pakistan. Hosseini and Hajizadeh (2011) designed species- specific primers to identify the three most common mealybug species on ornamental plants *viz*., *P. citri*, *Pseudococcus vibruni* and *Pseudococcus comstocki* from Iran. Abd-Rabou *et al*. (2012) studied the diversity in mealybug pest species in Egypt and France by combining morphological taxonomy based studies and three DNA markers *viz.,* 28S-D2, cytochrome oxidase I and internal transcribed spacer 2 and found genetic variation among the mealybugs thought to be belonging to the same group. Beltra *et al*. (2012) identified ten species of mealybugs from 33 mealybug samples collected, by amplification of universal barcode region mtCO1 from Spain.

Correa *et al*. (2012) characterized the mealybugs infesting the vineyards of Chile using mtCO1 and ITS 2 markers. Pacheco *et al*. (2014) conducted morphological and molecular characterization of mealybugs from the vineyards of Brazil and identified major mealybug pests of grapes by amplifying five DNA loci.

Wang *et al*. (2016) carried out DNA barcoding of mealybugs from Mainland China to assist morphological taxonomy and tested the efficacy of two molecular markers, mtCO1 and large ribosomal subunit gene (28S) and have setup Barcode library of mealybugs for rapid identification of mealybug pest of China. Assefa and Malindzisa (2018) conducted molecular characterization of mealybugs for the first time from Switzerland and identified major mealybug species infesting the ornamental and other wild hosts using the mtCO1

locus. Zheng *et al*. (2018) developed a tool for identification of the mealybugs a species level using short standard strand of sequences as labels for a species.

2.3.2 DNA barcoding of ants

Jansen *et al*. (2008) studied the identity of undescribed *Myrmica* ant specimens by employing DNA barcoding of the mtCO1 gene. Barcodes for sixteen species of ants by amplifying 658 bp region of mitochondrial cytochrome oxidase one gene was generated by Ojha *et al*. (2014) and they came to the conclusion that DNA barcoding is quick and reliable tool for species identification. Kouakou *et al*. (2017) identified invasive ant species *Solenopsis geminate* Fabricius from West Africa for the first time employing DNA barcoding methods. DNA barcoding for identifying aphid associated ants from the subtropical area of southern China by amplification of mtCO1 gene was carried out by Siddiqui *et al*. (2019). Rasool *et al*. (2020) utilized DNA barcoding to characterize *Solenopsis saudiensis* Sharaf and Aldawood, a species of fire ant collected from the date palm grooves of Saudi Arabia by amplification of mtCO1 gene.

2.4 Seasonal incidence of mealybugs

Raut *et al*. (2013) came to the conclusion that population of mealybugs infesting custard apple cv. Balanagar showed highly significant correlation with maximum temperature whereas, had a significant negative correlation with increasing relative humidity. Studies conducted by Debojith *et al*. (2013) revealed that the population of root mealybug, *Paraputo* sp. was very low during the cooler December to January and tends to rise with increase in temperature and relative humidity and slowly decline after the receipt of rains. The peak population was observed in August. According to Firake *et al*. (2015), the infestation of mealybug *F. polysperes* started in the month of August and reached its peak during January.

Nebie *et al*. (2016) came to a conclusion that the population of mango mealybug *Rastrococcus invadens* (Williams) increased during the wet season than during dry season and there was a significant correlation of population with the temperature, rainfall and

relative humidity in Western Burkina Faso. Population of mealybug *Maconellicoccus hirsutus* (Green) on grapes increased from January to March and lowest population was observed during July, the study also points out that relative humidity had a significant negative impact on the mealybug population buildup besides this the temperature and sunshine hours had a significant positive correlation with the pest population (Angu *et al.,* 2017).

Chauhan *et al*. (2017) recorded that infestation of mealybugs on *Bt* cotton commenced from September and population persisted till the harvest of the crop in Gujarat. The population of *P. solenopsis* was maximum during December and January (Sahu *et al*., 2017). Correlation of population with weather parameters showed a highly significant negative correlation with minimum and maximum temperatures. Swathi (2018) reported that the population of *F. polysperes* on the roots of black pepper increased during September and the peak population was attained in October in Chikkamagaluru district of Karnataka.

Zia and Haseeb (2019) witnessed that the mealybug *P. solenopsis* attained peak population during the last fortnight of August on okra from Uttar Pradesh, the study also showed that the population of mealybugs showed a negative correlation with maximum temperature whereas, positive correlation with relative humidity. The population of the mealybug *P. solenopsis* showed highly significant a positive correlation with maximum temperature at Giza during summer season whereas in Qalyubia, population showed a highly significant correlation with relative humidity during nili season from Egypt (Elbahrawy *et al*., 2020).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study on 'Diversity of root mealybugs of Kerala' was carried out at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara from 2018 to 2020. This study aimed at analyzing the diversity of mealybugs attacking the roots of different crop plants and also to study the association of ant species with these mealybugs. The materials and methods adopted for experiments conducted to achieve the objectives are given below.

3.1 DOCUMENTATION OF ROOTMEALYBUGS AND ASSOCIATED ANTS

Purposive sampling surveys were conducted during 2018- 2020 in Kasaragod, Wayanad, Kannur, Wayanad, Thrissur, Ernakulam and Idukki districts of Kerala. The main crop as well as weeds present in the field were observed during the survey for the presence of root mealybugs and associated ants. Details of areas covered along with GPS locations and host plants are furnished in Annexure I, Fig 1 and Plate 1.

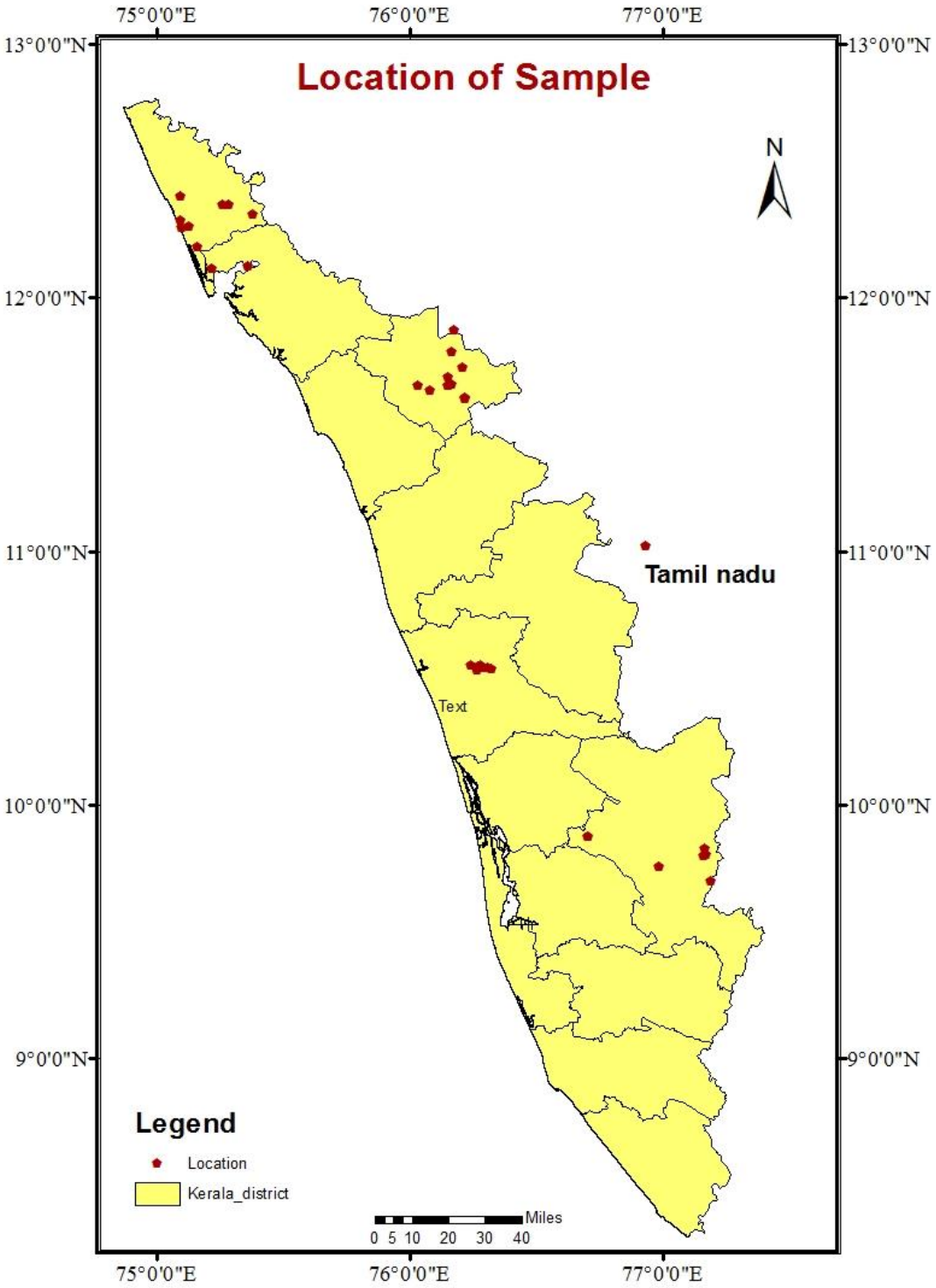
3.1.1 Sample collection

Infested plants were recognized by their sickly appearance and also by the presence of ants at the collar region. The soil present around the roots of the plants was carefully removed and the root mealybugs and associated ants were carefully collected with camel hair brush into separate microcentrifuge tubes containing ethyl alcohol (90 %) and were labeled for further processing. Distinct accession numbers were also given to the samples collected (Table. 1).

3.1.2 Assessment of soil physio-chemical properties

A soil sample of 500 g was collected from the field to check the physio-chemical properties of soil such as soil type, soil moisture, pH which was estimated in the laboratory.

Fig 1. Study area and locations surveyed



Parappa

Periye

Chaturakinar

Vellanikkara

Kannara

Plate 1. Survey and collection of specimens from different locations of Kerala

Table 1. Details of survey locations and host plants covered in the study with accession numbers

Sl. No.	District	Location	Host plants	Accession number of mealybugs	Accession numbers of ants
1.	Kasaragod	Parappa	Black pepper (*Piper nigrum*)	Blp1kas	Blp1kasant
2.		Chaturakinar	Banana (*Musa* sp.)	Ban1kas	Ban1kasant
3.		Periye	Black pepper (*Piper nigrum*)	Blp2kas	Blp2kasant
4.		Hosdurg	-	-	-
5.		Pilicode	-	-	-
6.		Chittarikal	-	-	-
7.		Vellarikund	-	-	-
8.	Kannur	Cherupuzha	-	-	-
9.		Panniyoor	Black pepper (*Piper nigrum*)	Blp1kan	Blp1kanant
10.		Pananpuzha	-	-	
11.		Pilathara	Black pepper (*Piper nigrum*)	Blp2kan	Blp2kanant
12.	Wayanad	Meenangadi	Nut grass (*Cyperus rotunus*) Para grass (*Brachiaria mutica*) Durian *(Durio zibethinus)*	Cyp2way Par1way Dur1way	-
13.		Kenichira	Avocado (*Persea americana*)	Avo2way	Avo2wayant
14.		Ambalavayal	Avocado (*Persea americana*) Mango (*Mangifera indica*)	Avo1way Man1way	Avo1wayant
15.		Kottathara	Coffee (*Coffea canephora*)	Cof1way	-
16.		Kambalakkad	Black pepper (*Piper nigrum*) Goat weed	Blp1way Goa1way	Blp1wayant

			(*Ageratum conyzoides*)		
17.		Kalpetta	-	-	-
18.		Pulpally	Nut grass (*Cyperus rotunus*)	Cyp1way	Cyp1wayant
19.	Thrissur	Vellanikkara	-	-	-
20.		Vellanikkara	Pineapple (*Ananas comosus*)	Pin1thr	Pin1thrant
21.		Thalikund	-	-	-
22.		Nadathara	-	-	-
23.		Kannara	-	-	-
24.	Ernakulam	Vazhakulam	-	-	-
25.	Kottayam	Kuruvilangad	-	-	-
26.	Idukki	Thannimood	Cardamom (*Elettaria cardamomum*)	Car1idu	Car1iduant
27.		Puttady	-	-	-
28.		Pampadumpara	-	-	-
29		Puliyanmala	-	-	-
30.		Todupuzha	-	-	-

The soil pH was estimated using pH meter and soil moisture was estimated by using a gravimetric method which involves drying 100 grams of the soil samples in a hot air oven at 60ºC till constant weight was achieved (Reynolds, 1970). The details of soil data collected from the survey areas are given in Annexure II.

The formula used for the calculation of soil moisture is given below:

$$\text{Soil moisture (\%)} = \frac{\text{Weight of fresh soil} - \text{Weight of oven-dried soil}}{\text{Weight of dry soil}} X\ 100$$

3.1.3 Morphological identification of Root Mealybugs

The specimens obtained from the field were brought to the laboratory and transferred into new clean vials and labelled. Adult mealybugs were carefully placed into a new vial and were sent for identification to Dr. Sunil Joshi, Principal Scientist, and Head of the Division (In-charge), Division of Germplasm collection and Characterization, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, Karnataka.

3.1.4 Morphological identification of ants

The field-collected specimens were brought back to the laboratory and were transferred into the new clean vial and labelled. Ants were carefully placed into a new vial and were sent for identification to Dr. Himender Bharthi, Department of Zoology and Environmental Sciences, Ant systematic and Molecular Biology lab, Punjabi University Patiala, Punjab.

3.1.5 Symptoms

The aerial, as well as subterranean symptoms of the infested crop plants and weeds, were noted. The symptoms included foliage yellowing and wilting, total plant drying, the presence of ant colonies at the collar region and symptoms below ground comprised the presence of white mealy wax on the roots, as well as discoloration at the feeding sites.

3.1.6 Collateral hosts

The weeds present in the field showing visual symptoms were uprooted for observing the presence of root mealybugs. Collection of samples was done in vials containing ethyl alcohol (90 %) and labelled for further processing.

3.2 MOLECULAR IDENTIFICATION OF MEALYBUGS AND ASSOCIATED ANT FAUNA

3.2.1 Isolation of genomic DNA from the mealybugs and ant specimens

Mealybug specimens were collected in vials containing ethyl alcohol (90 %) and stored in a deep freezer at -80 ºC. For DNA isolation, the specimens were retrieved from the individual vials separately. The DNA isolation from mealybug was carried out using CTAB method (Padmanabhan, 2018). The reagents used for the preparation of CTAB buffer are furnished in Annexure III.

- An adult female mealybug was picked from the storage vials with the help of a fine and soft paint brush into a sterile vial and was washed with double distilled sterile water. The mealybug was carefully placed on to a sterile tissue paper to remove excess moisture
- It was then transferred into a new 1.5 ml sterile microcentrifuge tube containing 100 µl preheated CTAB buffer with the help of a sterile brush. The mealybug was homogenized using a sterilized micropestle to form a uniform slurry and 600 µl of freshly prepared preheated CTAB buffer was added to the microcentrifuge tube and it was homogenized.
- The slurry was incubated at 65°C for 90 minutes with periodic gentle shaking in a water bath
- After the incubation, tubes were allowed to cool to room temperature for five minutes and 700 µl of freshly prepared pre-chilled chloroform: isoamyl alcohol (24:1) was added and mixed gently for five minutes. The tubes were centrifuged at 10,000 rpm for 10 minutes at room temperature
- After centrifugation, the top aqueous layer was transferred into a fresh labelled tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added again and vortexed for five minutes and centrifuged again at 10,000 rpm for 10 minutes at room temperature
- The top aqueous layer was collected, an equal volume of chilled isopropanol was added, mixed gently by inverting the tube. 30 µl of sodium acetate (3M, pH- 5.8) solution was added and incubated at -20°C for one hour

- After incubation the microcentrifuge tubes were centrifuged at 13,000 rpm for 10 minutes at 4 °C, the supernatant was removed retaining the pellet and 1 ml of ethanol (70 %) was added and centrifuge at 13,000 rpm for 10 minutes at 4 °C. Excess ethanol was removed and the tube was allowed to air dry
- The pellet was suspended in 25 µl nuclease free sterile distilled water

3.2.2 Assessment of DNA quality and quantity

3.2.2.1 *Spectrophotometric analysis*

The quality and quantity of the isolated DNA were determined by using IMPLEN® Nanophotometer. The instrument was calibrated using sterile distilled water (1 µl) as blank for zero absorbance and then DNA (2 µl) was subjected to analysis at 260 and 280 nm wavelengths and the absorbance was recorded. The absorbance $A_{260/280}$ was calculated to determine the quality of the DNA. The quantity of DNA recorded as nanograms per microlitre (ng/µl)

3.2.3 Amplification of mitochondrial cytochrome oxidase one (mtCO1 gene)

Polymerase chain reaction was carried out for the amplification of mtCO1 genes in an instrument called PCR Thermal cycler (BIO-RAD® thermal cycler) using specific primers (Table 2 and 3).

Table 2. Details of primers used for amplification of mtCO1 locus of mealybugs

Primer details	**Sequence 5'-3'**	**Reference**
- Forward primer (C1-J-2183 F)	CAACATTTATTTTGATTTTTTGG	Gullan *et al.* (2003)
- Reverse primer (C1-N-2568-R)	GCTACAACATAATAAGTATCATG	

Table 3. Details of primers used for amplification of mtCO1 locus of ants

Primer details	Sequence 5'-3'	Reference
- Forward primer (LCO 1490)	GGTCAACAAATCATAAGATATTGG	Ojha *et al*. (2013)
- Reverse primer (HCO 2198)	TAAACTTCAGGGTGACCAAAAAATCA	

3.2.2.4 Standardisation of annealing temperature for the primers

The reaction was carried out at varying temperatures to standardize the annealing temperature for both the set of primers. The temperatures used for standardization ranged from42, 43, 44, 45 degree Celsius for mealybug specific primer (C1-J-2183 F and C1-N-2568-R) and 53, 54, 55, 56 for the universal primer used for ants (LCO 1490, HCO 2198) and the best temperature was selected based on the quality of the band obtained in gel electrophoresis. The composition PCR reaction mixture for both ants and mealybugs is furnished in Table 4.

Table 4. PCR reaction mixture for mealybugs and ants

Component	Quantity (µl)/ reaction
Template DNA	2
PCR mastermix	10
Forward primer	0.8
Reverse primer	0.8
Distilled water	6.4
Total	20

The template DNA, forward and reverse primers, PCR master mix, Millipore® water were mixed thoroughly with the help of a mini spinner (TARSONS®) and were placed immediately into the thermal cycler. Gradient PCR was used for standardizing the annealing temperature and the conditions for thermal profiling of mtCO1 locus of mealybugs and ants (Table 5 and 6).

Table 5. Programme for PCR reaction of mealybugs

Steps	Temperature (°C)	Time (minutes)	
Initial denaturation	94	4	
Denaturation	94	30	35 cycles
Annealing	43.2	1	
Extension	72	2	
Final extension	72	10	

Table 6. Programme for PCR reaction for ant specimen

Steps	Temperature (°C)	Time (minutes)	
Initial denaturation	94	5	
Denaturation	94	1	35 cycles
Annealing	55	1	
Extension	72	1	
Final extension	72	10	

3.2.2.5 Gel documentation of the PCR products

Agarose gel electrophoresis

The PCR product was subjected to agarose gel electrophoresis to confirm the amplification of the mtCO1 region.

- The gel casting tray was wiped with ethyl alcohol (70 %) to remove any foreign material and was allowed to air dry
- 1X TAE buffer was prepared by diluting 50X TAE stock solution *i,e.,* one ml of 50X TAE buffer was added to 49 ml distilled water
- Agarose 0.6 gram was weighed and transferred into a microwave-safe conical flask containing 50 ml 1X TAE buffer and mixed well
- The solution was heated in a microwave for 1-3 minutes till the agarose completely dissolved
- Let the agarose solution cool to 50°C
- Add ethidium bromide (EtBr) to the solution so that the final concentration would be around 0.5µg/ml.
- The mixture is poured into the gel casting tray with well combs in place
- Let it solidify at room temperature for 30-40 minutes

Loading the samples and Running an agarose gel

- Once solidified the agarose gel was transferred into an electrophoresis tank with 1X TAE buffer such that the wells are at the cathode side (make sure that the gel is completely submerged in the buffer).
- PCR product (5 µl) was carefully loaded into the wells of agarose gel using a micropipette.
- DNA ladder,100 bp (3 µl) was also loaded into a well to determine the size of the PCR product
- The gel was run at 80 V until the dye had approximately covered three by fourth of the gel
- The device was turned off and disconnected from the electrodes and the gel was removed from the tank
- Visualization of amplified product and image capturing the of the gel was done uning gel documentation unit (UVITECH® Cambridge Gel doc.).

3.2.2.6 *Sequencing of the product*

The amplified product was sent to AgriGenome Labs Private. Limited, Cochin for sequencing using the forward and reverse primers.

3.3 DATA ANALYSIS USING *IN- SILICO* TOOLS

3.3.1 Sequence analysis and annotation

The forward and reverse sequences of each species had been assembled using the CAP3 sequence assembly programme to develop contigs (doua.prabi.fr/software/cap3). To verify the existence of stop codon using Mega 7, the assembled sequences were annotated.

3.3.2 Sequence homology analysis

The sequence homology of the contigs of each specimen was determined using a search tool by National Center for Biotechnology Information (NCBI) called Basic Local Alignment Search Tool for nucleotide (BLASTn). The query sequences were uploaded to NCBI BLASTn for nucleotides, (blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE= Nucleotide& PROGRAM=Blast). The programme show the deposited sequences which has similarity with the query sequence and also gives percent sequence identity, E value and query cover with the help of which species determination can be carried out.

3.4.5 PHYLOGENY TREE CONSTRUCTION

The software MEGA X was utilized for construction of phylogenetic tree. In the study, mitochondrial CO1 sequences of nine accessions of mealybugs were generated and mtCO1 sequences of mealybugs species were retrieved from NCBI database. *Coccus viridis* was chosen as an outgroup. The phylogeny tree was constructed using the “Phylogeny” tool of the software using the sequences.

3.4.6. Submission to Barcode of Life Data System

A total of 10 mtCO1 sequences generated in the study representing eight mealybug species and one ant species were submitted to Barcode of Life Data Systems (BOLD) for generating barcodes.

3.5 SEASONAL INCIDENCE OF ROOT MEALYBUGS

The seasonal incidence of root mealybug was studied at an infested black pepper field in Balal grama panchayath, Kasaragod, Kerala from March 2019 to February 2020. The mealybug species present in the field was identified as *F. polysperes*. Three vines were selected and kept free from insecticidal application during the period of study. Observation on the number of root mealybugs present on 15 cm root length at monthly intervals were recorded. The weather data *viz.,* maximum and minimum soil temperature, maximum and minimum relative humidity, rainfall and number of rainy days were obtained from the nearest meteorological station at the Regional Agricultural Research Station (RARS), Pilicode (Annexure IV).

3.5.1 Correlation of root mealybug population with weather parameters

The relationship between the weather parameters *viz.,* maximum and minimum soil temperature, maximum and minimum relative humidity, rainfall and number of rainy days and root mealybug population was analyzed by correlation analysis using IBM® SPSS® software.

RESULTS

4. RESULTS

A study on 'Diversity of root mealybugs of Kerala' was carried out at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara to understand the diversity of root mealybugs and associated ants from different crops of Kerala. The results of the study are presented in detail in this chapter.

4.1. SURVEY AND COLLECTION OF ROOT MEALYBUGS AND ASSOCIATED ANTS

Purposive sampling surveys were carried out at different districts of Kerala covering 30 locations and 11 host plants. Root mealybugs associated with crop plants and weeds *viz.,* black pepper, banana, cardamom, pineapple, coffee, avocado, mango, durian, para grass, nut grass and goat weed were collected from the root zone and stored in vials containing ethanol (90 %). The ants associated with the root mealybugs were collected separately in vials containing (90 %) ethanol. Specific accession numbers were given to the collected samples. A total of 17 mealybugs and 11 ant samples were obtained and preserved in the present study. Mealybug infestation was reported from three locations at Kasaragod, two locations at Kannur, six locations at Wayanad, two locations at Thrissur and one location at Idukki (Table 7).

4.2. MORPHOLOGICAL IDENTIFICATION OF ROOT MEALYBUGS

The study of slide-mounted specimens revealed the presence of eight species of root mealybugs associated with eleven species of host plants. The identification report is presented in Table 8.

4.2.1 Formicococcus polysperes

The adult females with broadly oval to almost spherical body and was covered with white waxy powder coating with clearly visible body segmentation. The wax filaments arising laterally in the anterior region were short whereas, in the posterior region, they were

slightly longer. The mealybug was collected from the roots of black pepper, banana, avocado and goat weed (Plate 2).

4.2.2 Formicococcus lingnani

The adult females were narrowly oval and are dorso-ventrally flattened . The mealy coating was present on pinkish body with prominent body segmentation. The wax filaments arising laterally were shorter anteriorly but were longer at the posterior end. The mealybugs were collected from the roots of nut grass (Plate 2).

4.2.3 Formicococcus mangiferacola

The adult females were oval, round and plumpy with a white waxy coating on the dorsal surface and the intersegmental lines were prominent. The lateral wax filaments were short at the anterior segment whereas in the posterior region, they were comparatively longer. The root mealybug was collected from the roots of mango seedlings (Plate 3).

4.2.4 Planococcus lilacinus

The adult females were broadly oval with a mealy covering on the surface of the body (Plate 3). The lateral wax filaments were prominent and more prolonged at the posterior end of the mealybugs. Coffee roots were seen harbouring *P. lilacinus*.

4.2.5 Planococcus sp. on black pepper

The adult females were oval, dorso-ventrally flattened, the derm was yellowish and was covered by mealy coating (Plate 4). The lateral wax filaments were of the same size anteriorly, but the last pair of posterior filaments were longer comparatively. The mealybugs were collected from the roots of black pepper.

4.2.6 Planococcus sp. on durian

The adult females were oval and were covered with mealy coating on the dark brownish coloured body (Plate 4). The lateral wax filaments were short and stout and were

present on all the segments. The mealybugs were collected from the roots and collar region of durian seedlings.

4.2.7 Dysmicoccus brevipes

The adult females were broadly oval with pinkish body covered by the mealy coating. The body segmentation was clear and prominent (Plate 4). The waxy filaments were conspicuous and with a slight increase in length at the posterior end of the mealybugs. The mealybugs were collected from the roots of pineapple and nut grass.

4.2.8 Antonina graminis

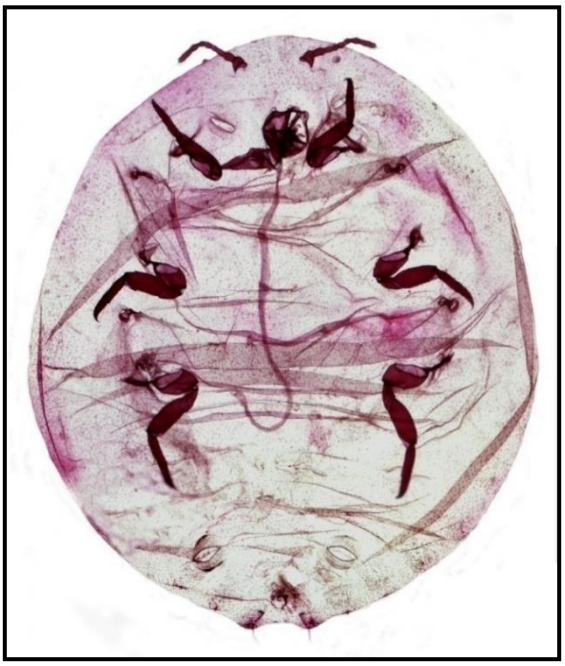
The adult females were broadly oval to circular, with heavy sclerotization throughout. The body was partially covered with mealy wax, and the sclerotized derm appeared to brownish red (Plate 5). The lateral wax filaments were not observed in the adult stage. These mealybugs were collected from the roots of para grass.

4.2.9 Xenococcus annandalei

The adult mealybugs were cream coloured with prominent antenna and well-developed legs. The abdomen was broad at the centre and narrowed down at the anterior and posterior ends. The mealy coating was absent (Plate 5). The mealybugs were obtained from the roots of cardamom.

Table 7. Details of locations of survey and host plants

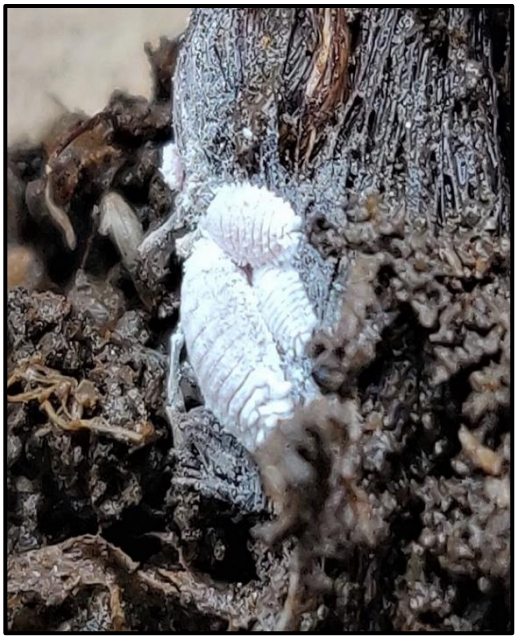
Sl. No.	District	Location	Host plants	Root mealybug infestation Present/absent
1.	Kasaragod	Parappa	Black pepper (*Piper nigrum*)	Present
2.		Chaturakinar	Banana (*Musa* sp.)	Present
3.		Periye	Black pepper (*Piper nigrum*)	Present
4.		Hosdurg	-	-
5.		Pilicode	-	-
6.		Chittarikal	-	-
7.		Vellarikund	-	-
8.	Kannur	Cherupuzha	-	-
9.		Panniyoor	Black pepper (*Piper nigrum*)	Present
10.		Pananpuzha	-	-
11.		Pilathara	Black pepper (*Piper nigrum*)	Present
12.	Wayanad	Meenangadi	Nut grass (*Cyperus rotunus*) Para grass (*Brachiaria mutica*) Durian *(Durio zibethinus)*	Present
13.		Kenichira	Avocado (*Persea americana*)	Present
14.		Ambalavayal	Avocado (*Persea americana*) Mango (*Mangifera indica*)	Present
15.		Kottathara	Coffee (*Coffea canephora*)	Present
16.		Kambalakkad	Black pepper (*Piper nigrum*) Goat weed (*Ageratum conyzoides*)	Present
17.		Kalpetta	-	-
18.		Pulpally	Nut grass (*Cyperus rotunus*)	Present



a. Adult female of *Formicococcus polysperes*

b. *Formicococcus polysperes*

c. a. Adult female of *Formicococcus lingnani*



d. *Formicococcus lingnani*

Plate 2. Species of *Formicococcus*

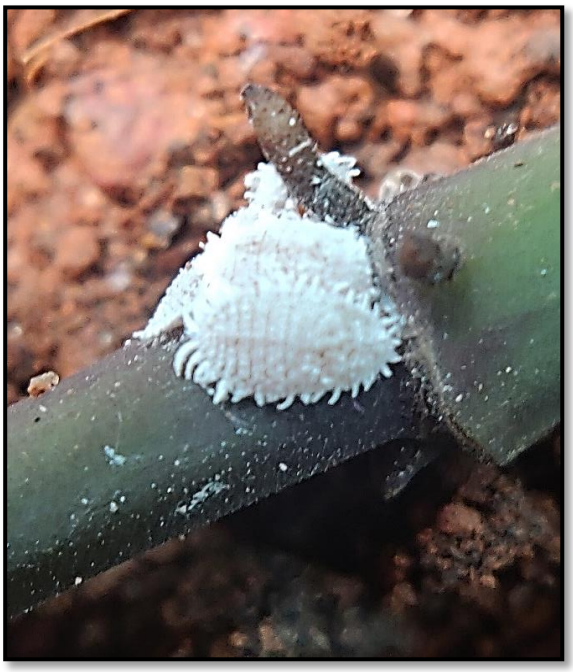
a. Adult female of *Formicococcus mangiferacola*

b. *Formicococcus mangiferacola*

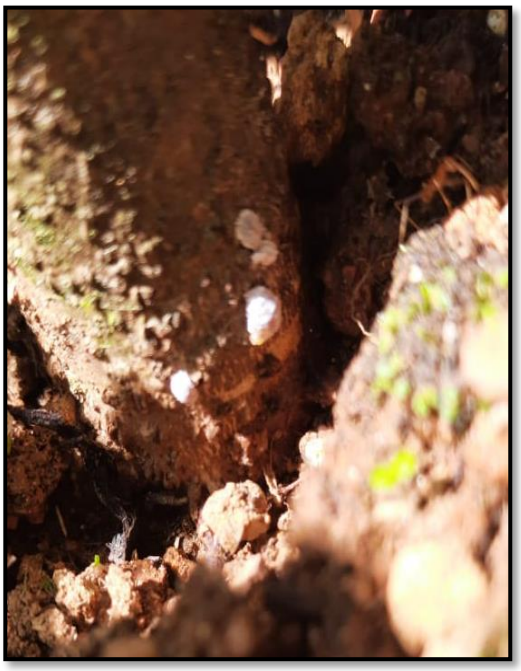
c. Adult female of *Planococcus lilacnus*

d. *Planococcus lilacnus*

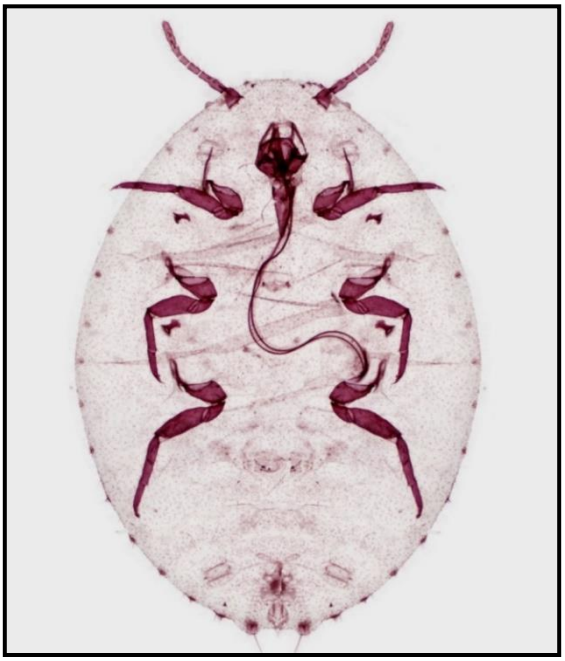
Plate 3. Species of *Formicococcus* and *Planococcus*



a. *Planococcus* sp. on black pepper



b. *Planococcus* sp. on durian



c. Adult female of *Dysmicoccus brevipes*

d. *Dysmicoccus brevipes*

Plate 4. Species of *Planococcus* and *Dysmicoccus*

a. Adult female of *Antonina graminis*

b. *Antonina graminis*

c. Adult female of *Xenococcus annandalei*

d. *Xenococcus annandalei*

Plate 5. Species of *Antonina* and *Xenococcus*

19.	Thrissur	Vellanikkara	-	-
20.		Vellanikkara	Pineapple (*Ananas comosus*)	Present
21.		Thalikund	-	-
22.		Nadathara	-	-
23.		Kannara	-	-
24.	Ernakulam	Vazhakulam	-	-
25.	Kottayam	Kuruvilangad	-	-
26.	Idukki	Thannimood	Cardamom (*Elettaria cardamomum*)	Present
27.		Puttady	-	-
28.		Pampadumpara	-	-
29		Puliyanmala	-	-
30.		Todupuzha	-	-

Table 8. Morphological identification of root mealybugs

Sl. No.	Accession number	Species
1.	Blp1kas	*Formicococcus polysperes* Williams
2.	Ban1kas	*Formicococcus polysperes* Williams
3.	Blp2kas	*Formicococcus polysperes* Williams
4.	Blp2kan	*Formicococcus polysperes* Williams
5.	Blp1kan	*Planococcus* sp. Ferris
6.	Cyp2way	*Dysmicoccus brevipes*Cockerell
7.	Par1way	*Antonina graminis* Maskell
8.	Dur1way	*Planococcus* sp. Ferris
9.	Blp1way	*Formicococcus polysperes* Williams
10.	Man1way	*Formicococcus mangiferacola* Williams
11.	Avo1way	*Formicococcus polysperes* Williams

12.	Cof1way	*Planococcus lilacinus* Cockerell
13.	Avo2way	*Formicococcus polysperes* Williams
14.	Cyp1way	*Formicococcus lingnani* Ferris
15.	Goa1way	*Formicococcus polysperes* Williams
16.	Pin1thr	*Dysmicoccus brevipes* Cockerell
17.	Car1idu	*Xenococcus annandalei* Silvestri

4.3 SYMPTOMS OF MEALYBUG INFESTATION ON DIFFERENT HOST PLANTS

4.3.1 Black pepper

The black pepper vine showed yellowing of the leaves during initial infestation, further upon persistent infestation, drying and defoliation of the leaves were observed. The turgidity of the plant was lost. Finally, complete wilting was observed. Another indication of the mealybug infestation was the presence of ants near the collar region of the plant. The presence of waxy mealy matter was observed on the roots as well as the collar region. Dark discoloration was also observed at the feeding site resulting in the blackening of roots. Mealybug colonies could be observed on the roots and at collar region. In the case of *Planococcus* sp. population could also be seen at the nodes from where roots were arising (Plate 6).

4.3.2 Banana

In banana plants, the symptoms ranged from complete drying up of the plants, presence of wilting symptoms and also marginal necrosis and drying of the leaves. When the subterranean parts of plants showing yellowing symptoms were explored, there was discoloration of the roots and surface of corms The mealybug colonies were present in between the dry scales of the corms and there was rotting off the corms due to secondary infection by soil borne pathogens (Plate 7).

a. Field view

b. Severely wilted black pepper vine

c. Infestation at the collar region

c. Infestation on the roots

Plate 6. Symptoms on black pepper

a. Field view

b. Severely infested plant

c. Rotting due to secondary infection

d. Mealybug infestation on corms

Plate 7. Symptoms on banana

4.3.3 Avocado

The symptoms exhibited by avocado plants infested with root mealybugs involved the wilted appearance of the plants, presence of ants in the basal region. The mealybugs could be seen infesting the subterranean plant parts such as cotyledons and tender roots of young seedlings. In severe case complete drying of plants was observed (Plate 8).

4.3.4 Cardamom

The cardamom clump showed yellowing wilting and drying of leaves. Close observation on the root zone revealed the presence of both nymphs and adults on the roots as well as on soil. The root mealybugs were seen congregating on the tender feeder roots of the host plant. In addition, numerous ants were present in the root zones and ant colonies could be observed around the roots. Some ants were seen carrying the mealybugs when disturbed (Plate 9).

4.3.5 Coffee

In coffee orchard mealybug infested showed yellowing of the leaves, wilting and loss of vigor. The presence of mealybug colonies at the collar region and also on the roots were seen when the soil around the stem and roots were removed (Plate 9).

4.3.6 Mango

The seedlings of mango appeared completely dried and the plants were brittle and could easily break when slight pressure was applied. On uprooting of the plant, mealybugs were present feeding on the young roots (Plate 10).

4.3.7 Durian

The durian seedlings showed yellowing of leaves and loss of vigour. The occurrence of mealybugs could be seen at the collar region and also on roots (Plate 10).

4.3.8 Pineapple

The plants were exhibiting slight yellowing on the older leaves and also pinkish tinge was also observed among some plants in the field. The plants showing yellowing symptoms when uprooted, colonies of mealybugs could be seen at the basal stem, tender roots of the plants. Ants were also observed at the rootzone attending the mealybugs (Plate 11).

4.3.9 Weed hosts

The weed plants present in the vicinity of the main crop were uprooted randomly and observed for the presence of root mealybugs. Some of the weed plants infested with root mealybugs showed symptoms for example nutgrass infested with root mealybug *F. lingnani* showed yellowing symptoms whereas, para grass and goat weed infested with root mealybugs did not show any external symptoms of wilting or yellowing. The plants appeared completely healthy (Plate 11 and 12).

4.4. ROOT MEALYBUG DIVERSITY IN DIFFERENT AGRICULTURAL ECOSYSTEMS OF KERALA

4.4.1. Diversity of root mealybugs

The study revealed the presence of eight species of root mealybugs belonging to five genera *viz*., *Formicococcus* Takahashi*, Planococcus* Ferris *, Dysmicoccus* Ferris*, Antonina* Signoret and *Xenococcus* Silvestri. Maximum diversity was found in the genus *Formicococcus,* representing three species *viz*., *Formicococcus polysperes* Williams, *F. lingnani* Ferris and *F. mangiferacola* Williams. The genus *Planococcus* was represented by *Planococcus lilacinus* and *Planococcus* sp. The genera *Dysmicoccus*, *Antonina* and *Xenococcus* were represented by one species each, *viz*., *Dysmicoccus brevipes* Cockerell, *Antonina graminis* Maskell and *Xenococcus annandalei* Silvestri. Three species *viz., F. lingnani*, *F. mangiferacola* and *A. graminis* were recorded for the first time from Kerala. (Table 9)

a. Wilted avocado seedling

b. Infestation of mealybugs on roots

c. Infestation of mealybugs on cotyledons

Plate 8. Symptoms on avocado

a. Dried clump

b. Infestation on the roots

c. Yellowing and wilting of the foliage

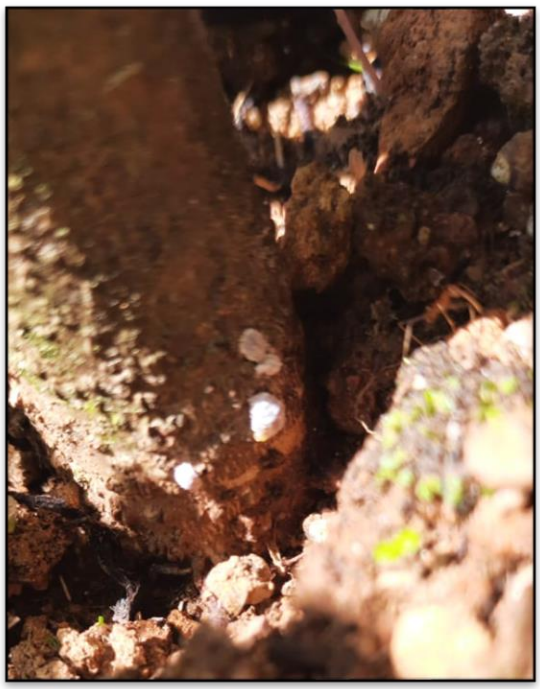
d. Infestation at collar region

Plate 9. Symptoms on cardamom and coffee

a. Wilted mango seedling

b. Infestation of mealybugs on roots

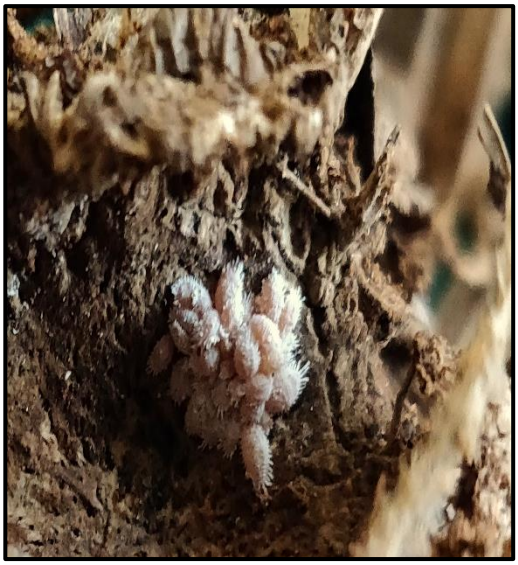
c. Wilted durian seedlings



d. Infestation at the collar region

Plate 10. Symptoms on mango and durian

a. Yellowing of the leaves



b. Infestation on the roots

c. No external symptoms

d. Infestation on the roots

Plate 11. Symptoms on pineapple and nut grass

a. Yellowing of the foliage

b. Infestation on roots and collar region

c. No external symptoms on goat weed

d. Infestation on roots

Plate 12. Symptoms on nutgrass and goat weed

Table 9. Diversity of root mealybugs infesting various host plants in Kerala

Sl. No.	Host plants	Mealybug species
1	Black pepper, banana, avocado, goat weed	*Formicococcus polysperes*
2	Nut grass	*Formicococcus lingnani*
3	Mango	*Formicococcus mangiferacola*
4	Nut grass, pineapple	*Dysmicoccus brevipes*
5	Para grass	*Antonina graminis*
6	Coffee	*Plannococcus lilacinus*
7	Durian, black pepper	*Planococcus* sp.
8	Cardamom	*Xenococcus annandalei*

4.3.2. Host range of root mealybugs

Among the plants surveyed black pepper and nutgrass were seen to be infested with two species of mealybugs. Black pepper was associated with *F. polysperes* and *Planococcus* sp. and nut grass with *F. lingnani* and *D. brevipes*. All the other host plants were infested with only one species of mealybug each. Fruit plants like banana and avocado and the common weed species *Ageratum conyzoides* were seen harbouring *F. polysperes*. Coffee roots were seen infested with *P. lilacinus.* Another tropical fruit crop, durian was infested by the root mealybug, *Planococcus* sp. whereas pineapple was found to be associated with *D. brevipes*. Queen of spices, cardamom was severely infested by the root mealybug, *X. annandalei.*

The root mealybug, *F. polysperes* had a wider host range *viz*., black pepper, banana, avocado and goat weed. Pineapple mealybug, *D. brevipes* was recorded on pineapple and nut grass while *Planococcus* sp. was recorded on durian and black pepper. The hypogaeic mealybugs, *F. lingnani*, *F. mangiferacola*, *A. graminis*, *P. lilacinus* and *X. annandalei* were recorded from single host plant each *viz*., nut grass, mango, para grass,

coffee and cardamom respectively (Table 10). From the table it is obvious that the root mealybug, *F. polysperes* was associated with maximum number of host plants belonging to four different families *viz*., Piperaceae, Musaceae, Lauraceae and Asteraceae respectively. Subterranean mealybugs, *D. brevipes* and *Planococcus* sp were recorded from two host plants each belonging to two different families like Cyperaceae and Bromiliaceae and Malvaceae and Piperaceae respectively.

Table 10. Host range of root mealybug species

Sl. No.	**Mealybug species**	**Number of host plants**	**Host plants**	**Family**
1.	*Formicococcus polysperes*	4	Black pepper, banana, avocado, goat weed,	Piperaceae Musaceae, Lauraceae and Asteraceae
2.	*Formicococcus lingnani*	1	Nut grass	Cyperaceae
3.	*Formicococcus mangiferacola*	1	Mango	Anacardiaceae
4.	*Dysmicoccus brevipes*	2	Nut grass, pineapple	Cyperaceae and Bromiliaceae
5.	*Antonina graminis*	1	Para grass	Poaceae
6.	*Plannococcus lilacinus*	1	Coffee	Rubiaceae
7.	*Planococcus* sp.	2	Durian, black pepper	Malvaceae and Piperaceae
8.	*Xenococcus annandalei*	1	Cardamom	Zingiberaceae

4.4. MORPHOLOGICAL IDENTIFICATION OF ANTS

The morphological study of the specimens revealed that seven species of ants were associated with diverse species of root mealybugs from different locations of Kerala. The identification report of ants is presented below (Table 11). A total of 11 ant samples were collected from various places and got identified.

4.5. ANTS ASSOCIATED WITH ROOT MEALYBUGS

Four species of ants *viz., Tapionoma indicum* Forel, *Nylandria indica* Forel. *Myrmicaria brunnea* Saunders, W. W and *Crematogaster rogenhoferi* Mayr were associated with root mealybug *F. polysperes* from various locations of Kerala (Plate 13). The ant species, *N. indica* was associated with root mealybug *F. lingnani* from Pulpally of Wayanad district. The ant species, *C. affinis* was associated with root mealybug *D. brevipes*, from Vellanikkara of Thrissur district (Plate 14). The ant species, *P. longicornis* was associated with *Planococcus* sp. on black pepper from Kannur district (Plate 15). The ant, *A. acutiventris* was associated *X. annandalei* with from Idukki district (Plate 15). The ant, *N. indica* was seen associated with two subterranean mealybugs *viz*., *F. polysperes* and *F. lingnani* respectively. All the remaining ant species were associated with only single root mealybug species respectively (Table 12).

Table 11. Morphological identification of ants

Sl. No.	Accession number	Species
1.	Blp1kasant	*Tapinoma indicum* Forel
2.	Ban1kasant	*Tapinoma indicum* Forel
3.	Blp2kasant	*Nylanderia indica* Forel
4.	Blp1kanant	*Nylanderia indica* Forel
5.	Blp2kanant	*Paratrechina longicornis* Latreille
6.	Avo2wayant	*Myrmicaria brunnea* Saunders, W.W
7.	Avo1wayant	*Nylanderia indica* Forel
8.	Blp1wayant	*Crematogaster rogenhoferi* Mayr
9.	Cyp1wayant	*Nylanderia indica* Forel
10.	Pin1thrant	*Carebara affinis* Emery
11.	Car1iduant	*Acropyga acutiventris* Roger

Table 12. Ants associated with mealybugs

Sl. No.	Mealybug species	Ant species
1.	*Formicococcus polysperes*	*Nylanderia indica*
		Tapinoma indicum
		Myrmicaria brunnea
		Crematogaster rogenhoferi
2.	*Formicococcus lingnani*	*Nylanderia indica*
3.	*Dysmicoccus brevipes*	*Carebara affinis*
4.	*Planococcus* sp.	*Paratrechina longicornis*
5.	*Xenococcus annandalei*	*Acropyga acutiventris*

4.6. MOLECULAR CHARACTERIZATION OF ROOT MEALYBUGS AND ANTS

4.6.1. Isolation of genomic DNA

The genomic DNA of eight accessions of root mealybugs and one accession of ant, each representing different species were isolated using the modified CTAB method. The quantity and quality of the isolated DNA were analysed and the absorbance values were recorded at $A_{260/280}$. Absorbance values ranged between 1.9- 2.1, and the quantity of DNA ranged from 74-734 ng/µl. The quality and quantity of the isolated DNA recorded is provided in Table 13.

4.6.2. Amplification of barcode locus mtCO1

The universal barcode region or the mtCO1 region of the specimen was amplified using the primers specified for mealybugs and ants. The length of the amplified fragment was between 400-500 bp for the mealybugs and 700-800 bp for ants. The PCR products were assessed for amplification by 1.2 per cent agarose gel electrophoresis. Bands were formed in the region between 400-500 bp for the mealybugs and 700-800 bp for ants indicating amplification (Plate 16).

Tapinoma indicum

Nylanderia indica

Formicococcus polysperes

Myrmicaria brunnea

Crematogaster rogenhoferi

Plate 13. Ants associated with *Formicoccus polysperes*

Formicococcus lingnani

Nylandria indica

Dysmicoccus brevipes

Carebara affinis

Plate 14. Ant associated with *Formicococcus lingani* and *Dysmicoccus brevipes*

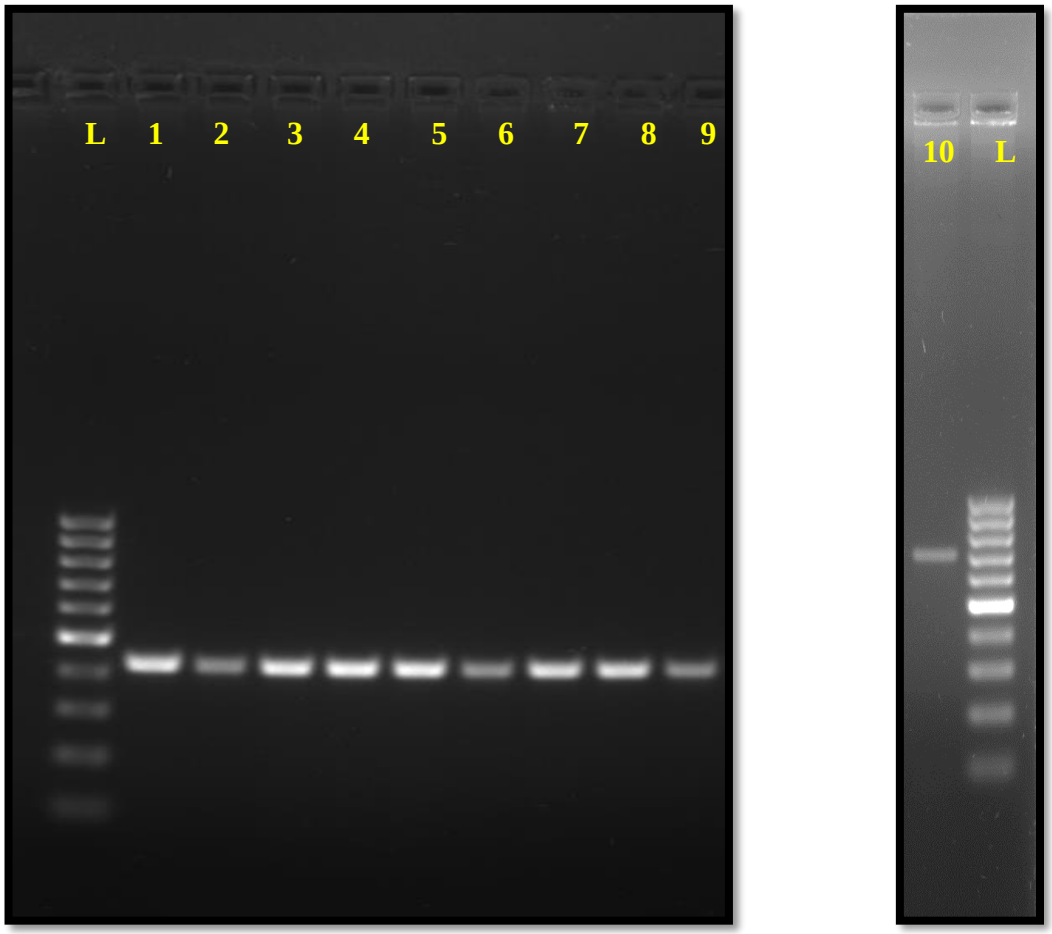
***Planococcus* sp. on black pepper**

Paratrechina longicornis

Xenococcus annadalei

Acropyga acutiventris

Plate 15. Ant associated *Planococcus* sp. and *Xenococcus annadalei*



a. amplification of mtCO1 of mealybugs **b. amplification of mtCO1 of ants**

L. Ladder (100bp), 1- Blp1kas; 2-Ban1kas; 3- Blp2kan; 4- Cyp2way; 5- par1way; 6-Man1way; 7-Cof1way; 8- Cyp1way; 9- Car1way; 10- Blp1wayant

Plate 16. Amplification of mtCO1 of mealybugs and ants

4.6.3. Sequencing of PCR product

The sequencing of the PCR products of all the accessions, which formed a single good band in gel electrophoresis, was outsourced to AgriGenome Labs. Pvt. Ltd., Cochin.

4.6.3.1 Sequence data of mealybugs and ants

F. polysperes (MW025198)
TGATTTTTTGGCCATCCTGAAGTATATATTTTAATCCTGCCTGGATTTGGAATTATATCT CAAATTATAAATCAAGAAAGTGGTAAAATAGAAATTTTTAGTAAAATTAATATAATTTT TGCTATAATTTCAATTGGAATTTTAGGATTTATTGTCTGAGCTCATCATATATTCACTAT TGGATTAGATATCGACACTCAATTATATTTTATATCAGCTACAATAATTATTGCTATCCC AACTAGAATTAAAATTTTTAGATGAATAATAACTTTAAATGGTAAAAAAATTCTCCACT CAACAATTAATTATTGATCTATAGGATTTATTATTATATTTACATTAGGAGGCCTAACA GGAATTATATTATCAAATTCAATTATTGATATTAATTTACATGATACCTAT
F. polysperes (MW025240)
TTGATTTTTTGGCCATCCTGAAGTATATATTTTAATCCTGCCTGGATTTGGAATTATATC TCAAATTATAAATCAAGAAAGTGGTAAAATAGAAATTTTTAGTAAAATTAATATAATTT TTGCTATAATTTCAATTGGAATTTTAGGATTTATTGTCTGAGCTCATCATATATTCACTA TTGGATTAGATATCGACACTCAATTATATTTTATATCAGCTACAATAATTATTGCTATCC CAACTAGAATTAAAATTTTTAGATGAATAATAACTTTAAATGGTAAAAAAATTCTCCAC TCAACAATTAATTATTGATCTATAGGATTTATTATTATATTTACATTAGGAGGCCTAAC AGGAATTATATTATCAAATTCAATTATTGATATTAATTTACATGATACCTAT
F. lingnani (MW027848)
TCAACATTTATTTTGATTTTTTGGTCATCCAGAAGTTTATATTTTAATTTTACCCGGTTT TGGAATTATATCACAAATTATAAATCAAGAAAGAGGAAAAATAGAAATTTTTAGAAAA ATTAATATAATTTTTGCTATAATTTCCATTGGAATTTTAGGATTTATTGTTTGAGCACAT CATATATTTACTATTGGATTAGATATTGATACACAATTATATTTCATATCAGCTACTATA ATTATTGCTATTCCTACAAGAATTAAAATTTTTAGTTGAATAATAACTTTAAATGGAAA AAAATATTTTTATTCAATTATTAATTTTTGATCCATAGGTTTTATTATTATATTTACCTTA GGTGGATTAACTGGAATTATTTTATCTAATTCAATTATTGATATTAATCTTCATGATACC TA
F. mangiferacola (SUB8185577)
ATTTTGATTTTTTGGCCATCCAGAAGTTTATATTCTTATTCTACCTGGATTTGGAGCTAT

ATCTCAAATTATAAATCAAGAAAGGGGAAAAATAGAAATTTTTAGTAAAATTAATATA ATTTTTGCTATAATTTCTATTGGTATTTTAGGTTTTATTGTTTGAGCCCATCACATATTCA CTATTGGATTAGATATTGATACACAATTATATTTTATATCAGCTACTATAATTATTGCTA TTCCAACTAGAATTAAAGTATTTAGATGAATAATAACTCTAAATGGAAAAATAATTATT AATTCATCAATTAATTTTTGATCTATTGGATTTATTATTATATTTACTCTAGGGGGATTA ACGGGAATCATTTTATCTAATTCTATTATTGATATTAATCTTCATGATACCTATAAGTA
D. brevipes (MW025235)
TCAACATTTATTTTGATTTTTTGGTCACCCTGAAGTTTATATTTTAATTTTACCAGGATT GCTACTACTAATAGGTATCATGAAGATTAATATCAATAATTGAATTAGATAAAATAAT ACCTGTTAAACCTCCTATTGTAAATATTATAATAAATCCTATAGATCATAATATAATAG ATGAATTTGAAATTTTTTTTCCATTTAAAGTTATTATTCAACTAAAAATTTTAATTCTTG TTGGAATAGCAATGATTATTGTAGCTATTATAAAATATAATTGAGTATCAATATCTAAT CCAATAGTAAATATATGATGAGCTCATACAATAAATCCTAAAATTCCAATAGAAATTA TAGCAAAAATTATATTAATTTTACTAAAAATTTCTAGTTTCCCACTTTCTTGATTTATAA TTTGAGATATAGCTCCA
A. graminis (MW025234)
TCAACATTTATTTTGATTTTTTGGTCATCCTGAAGTTTATATTTTAATTTTACCAGGATT TGGAGCTATATCACAAATTATAAATCAAGAAAGAGGAAAACTAGAAATTTTTAGAAAA ATTAATATAATTTTTGCAATAATTTCTATTGGTATTTTAGGATTTATTGTATGAGCTCAT CATATATTCACGATTGGCCTTGATATTGACACACAAATATATTTTATATCAGCTACAAT AATTATTGCTATTCCTACAAGAATTAAAATTTTTAGTTGAATAATAACTTTAAATGGAA AAAAAATTCTTTTTTCTTCAATTACATTATGATCAATAGGATTTATTATTATATTCACAC TTGGAGGTCTAACAGGAATTATTTTATCAAATTCTATTATTGATATTAATCTTCATGATA CCTAT
P. lilacinus (MW025236)
TTGATTTTTTGGACATCCTGAAGTTTACATTTTAATTTTACCAGGATTTGGAATAATATC TCAAATTATAAACCAAGAAAGAGGAAAAATAGAAATTTTTAGTAAAATTAATATAATT TTTGCAATAATTTCCATTGGAATTTTAGGTTTTATTGTTTGAGCTCATCATATATTTACT ATTGGATTAGACATTGACACTCAATTATATTTTATATCAGCTACAATAATTATTGCTATC CCTACTAGAATTAAAATTTTCAGATGAATAATAACTTTAAATGGAAAAAAAATTTTAA ATTCATCAATTAATTTTTGATCAATTGGATTTATTATTATATTTACTTTAGGGGGTTTAA CAGGTATCATTTTATCTAATTCAATTATTGATATTAACTTACATGATACCTAT
Planococcus sp. (SUB8185578)

TGATTTTTTGGACATCCAGAAGTTTATATTTTAATTTTACCAGGTTTTGGAACTATATCC CAAATTATAAATCAAGAAAGAGGAAAAATAGAAATTTTTAGTAAAATTAATATAATTT TTGCTATAATTTCAATTGGAATTTTAGGTTTTATTGTTTGAGCTCATCATATATTTACTA TCGGATTAGATATTGATACACAATTATATTTTATATCAGCTACAATAATTATTGCTATCC CTACAAGAATTAAAATCTTTAGATGAATAATAACTTTAAATGGTAAAAAAATTCTTAAT TCATCTATTAACTTTTGATCAATTGGATTCATCATTATATTTACATTAGGAGGATTAACT GGAATTATTTTATCAAATTCTATTATTGATATTAATCTTCATGATACCTA
X. annadalei (MW028133)
ACATTTATTTTGATTTTTTGGACACCCCGAAGTATACATTCTTATTCTTCCAGGATTTGG AATTATATCTCAAATTATAAACCAAGAAATAAACAAAAAAGAAATATTTAGAAAAATA AATATAATTTACGCTATAATCTCTATTGGCATTCTAGGATTTATCGTATGAGCTCACCAT ATATTTACTATTGGAATAGACATTAATTCACAATTTTACTTTATATCATCTACTATAATT ATTGCTATCCCTACAAGAATTAAAATCTTTAGATGAATAATCTCTATAAATAACAAAAA AAGAAACTTATCATCAATTTTTCTCTGATCAATTAGATTTATCTTAATATTTTCCATTGG AGGAATAACAGGTATTATTTTATCTAATTCAATTATTGATATTAACCTTCATGATACCT ATT
C. rogenhoferi (MW233048)
TTAAACTTCCAGGGTGACCAAAAAATCAGAATAAGTGTTGATATAAGATAGGGTCTCC TCCTCCTGAGGGGTCAAAGAAAGAGGTATTTAAATTACGGTCAGTTAGGAGTATGGTA ATAGCACCAGCAAGGACAGGTAAAGATAAAAGTAAAAGAATGGCGGTAATTAGAATA GATCATGTTAATAAATTAATTTTATCAAGAGAGAGAGATTTATGATGTATGTTTAAAAT AGTAGCAATAAAATTGATCGCACCTAAAATAGAGGATATACCTGCAATGTGAAGTGAA AAAATTGAAAGGTCAACTGAGGGGCCTCTATGGAAAATATTAGAGGCTAAGGGCGGAT AAATAGTTCATCCAGTACCTACTCCGGTATTAAGAAATCTACTAAGAAGAAGAAGGAG AATAGAGGGTGGTAGGAGTCAAAATCTTATATTGTTTATTCGGGGGTAAGCTATATCA GGTGAGCCTAATATTAAAGGTACCAAGAAATTTCCAAATCCTCCGATTATAAAGGGTA TAACTATAAAAAAAATTATAACAAAGGCATGCCCAGTAACAAGGACATTATAAATTTG GTCATTATAAATTAATGAATTGCATGAACCAAGTTCAAGTCGGATAATTATGCTTATAG AAGACCCGATTATTCCTGCTCAAATAGCAAAGATAAAGTAAAGAATTCCAATATCTTT AT

Table 13. Quality and quantity assessment of isolated DNA

Sl. No.	Accession number	$A_{260/280}$	Concentration (ng/ µl)
1	Blp1kas	2.06	158.20
2	Ban1kas	2.10	323.20
3	Blp2kan	2.13	432.05
4	Cyp2way	1.93	586.0
5	Par1way	2.08	734.45
6	Man1way	1.9	322.50
7	Cof1way	2.12	615.25
8	Cyp1way	2.10	416.15
9	Car1way	2.10	74.0
10	Blp1wayant	2.03	93.00

4.7. ANALYSIS OF MOLECULAR DATA USING *IN- SILICO* TOOLS

4.7.1. Sequence homology analysis of mealybugs and ants

The trimmed forward and reverse sequences were combined to form contig using CAP3 sequence assembler; Homology of the sequences was analyzed using Basic Local Alignment Search Tool for nucleotide (BLASTn) of NCBI database. The sequences which showed maximum percent identity, query cover and zero E value of those from the database were identified.

The sequences generated for *F. polysperes* and *P. lilacinus* showed 100 per cent similarity with that of the corresponding species and *Planococcus* sp. recorded from black pepper and *D. brevipes* showed 99.75 per cent similarity with *P. minor* and 98.9 per cent similarity with *D. brevipes respectively*. The ant species showed 95 per cent similarity with *Crematogaster* sp. The sequences of mealybug species *F. lingnani*, *F. mangiferacola*, *A. graminis* and *X. annandalei* were deposited for the first time in NCBI database hence did not show significant similarity (Table 14).

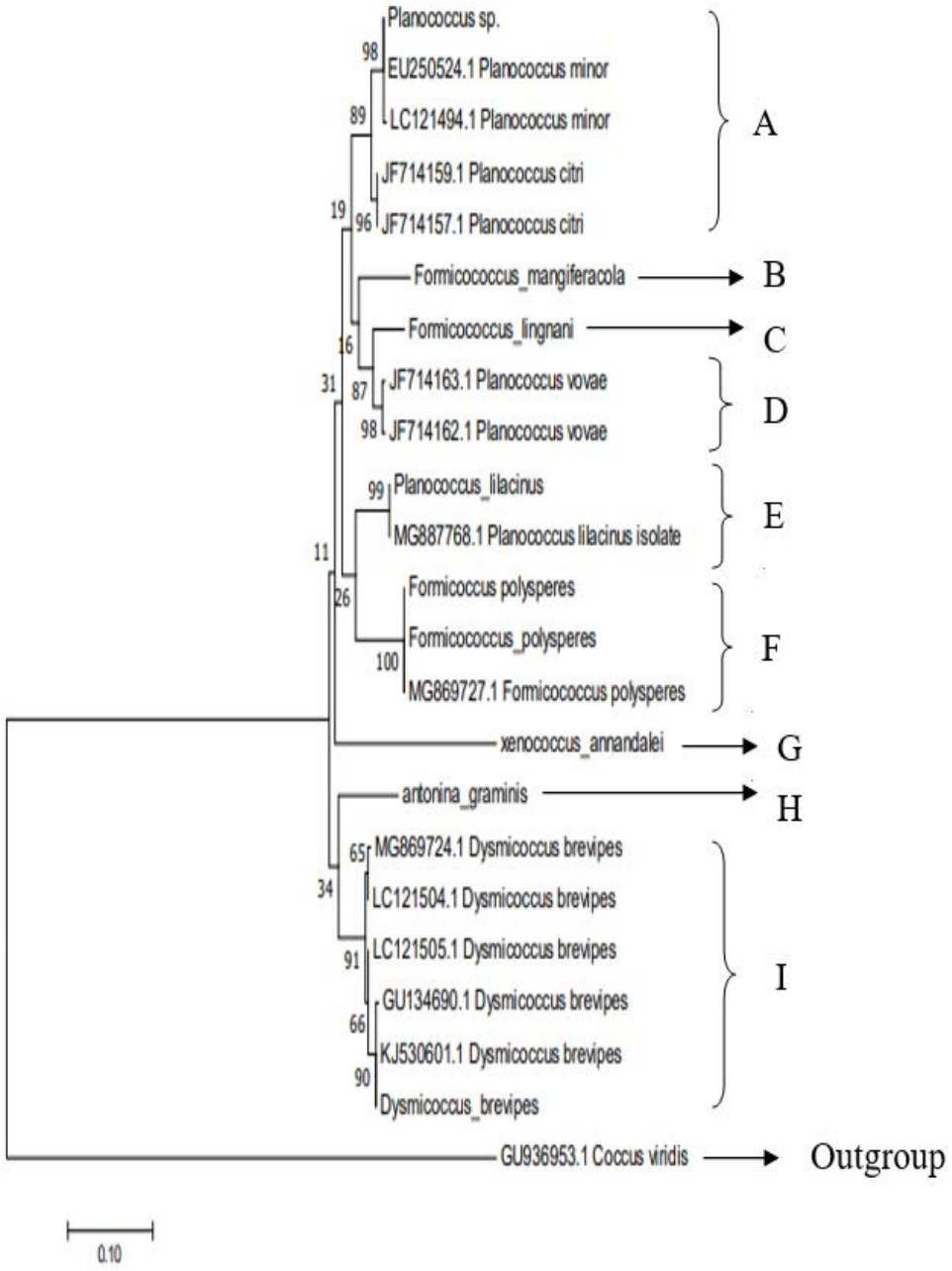
Table 14. Homology of the sequences generated in NCBI database

Sl. No.	Sample code	Insect species	Query coverage	Identity per cent	E value	Corresponding hits	Corresponding Species
1	Blp1kas	*Formicococcus polysperes*	100	100	0	MG869727.1	*Formicococcus polysperes*
2	Ban1kas	*Formicococcus polysperes*	100	100	2e-180	MG869727.1	*Formicococcus polysperes*
3	Man1way	*Formicococcus mangiferacola*	98	92.23	1e-162	JF714163.1*	*Planococcus vovae**
4	Cyp1way	*Formicococcus lingnani*	100	94.06	9e-179	JF714163.1*	*Planococcus vovae**
5	Cof1way	*Planococcus lilacinus*	100	100	0	MG887768.1	*Planococcus lilacinus*
6	Blp2kan	*Planococcus* sp.	99	99.75	0	LC121494.1	*Planococcus minor*
7	Par1way	*Antonina graminis*	100	91	3e-164	LC121505.1 *	*Dysmicoccus brevipes**
8	Cyp2way	*Dysmicoccus brevipes*	97	98.9	0	LC121505.1	*Dysmicoccus brevipes*
9	Car1idu	*Xenococcus annandalei*	100	83.37	4e-103	LC121504.1 *	*Dysmicoccus brevipes**

4.7.2 Phylogenetic tree

Phylogenetic tree was constructed using MEGA X software with maximum parsimony tree and bootstrap value of 1000. Nine mtCO1 sequences were generated in the study, and 13 sequences of mealybugs were retrieved from the NCBI database (Fig 2). The mtCO1 sequence of scale insect *Coccus viridis* (GU935963.1) was selected as an outgroup. In clade A, the mealybug identified as *Planococcus* sp. morphologically, showed the similarity of 98 per cent with retrieved accessions of *Planococcus minor.* In clade B and C, the mealybug was morphologically identified as *F. mangiferacola* and *F. lingnani*

Fig 2. Maximum parsimony tree with Bootstrap value 1000 indicating clustering of mtCO1 sequences of mealybugs



but there was no significant similarity with sequences deposited in NCBI. The sequences of these mealybug species were deposited in NCBI database for the first time in the present study. The clade B and C formed between clades A, D and E representing *P. minor*, *P. citri*, *P. vovae* and *P. lilacinus* showing more similarity with genus *Planococcus*.

In clade E, the mealybug identified as *P. lilacinus* shows 99 per cent similarity with that of retrieved accession of *P. lilacinus*. The clade F is represented by *F. polysperes* where the morphologically identified sequence showed cent per cent similarity with the retrieved sequence. The clade G and H are represented by *X. annandalei* and *A. graminis* respectively. Sequence similarity of the above mentioned mealybugs were not obtained with the retrieved sequences from NCBI. The sequences of these mealybugs were submitted for the first time to NCBI in the present study. In clade I, the mealybug identified morphologically as *D. brevipes* showed 90 per cent similarity with the retrieved sequence of the same species.

4.7.3 Sequence submission to BOLD

A total of nine sequences of mealybugs representing eight mealybug species and one sequence of ant which was obtained by amplification of a region of mtCO1 was submitted to Barcode of Life Data System (BOLD) to generate DNA barcodes. The process ID developed for submission of sequences in BOLD (Table 15). The illustrative DNA barcodes developed for a total of ten sequences (Fig 3).

4.8 SEASONAL INCIDENCE OF ROOT MEALYBUGS

The study on seasonal incidence of root mealybug, *F. polysperes* from March 2019 to February 2020 of root mealybug *F. polysperes* revealed that the mealybug population was present in the field throughout the study. The population of mealybugs increased in the rainy season and was highest in September 2019 (71.67 mealybugs / 15 cm root length) followed by August (63.34 mealybugs / 15 cm root length) and October (56.67 mealybugs / 15 cm root length) as the southwest monsoon subsides. The lowest population of root

mealybugs was observed in the summer months *i.e.,* March and April (4.3 and 4.0 mealybugs/15 cm root respectively) (Table 16).

Table 15. Process ID of the BOLD submission

Sl. No.	Accession no	Process ID	Species
1	Blp1kas	KAUEN001-20	*Formicococcus polysperes*
2	Ban1kas	KAUEN00`2-20	*Formicococcus polysperes*
3	Man1way	KAUEN003-20	*Formicococcus mangiferacola*
4	Cyp1way	KAUEN004-20	*Formicococcus lingnani*
5	Cof1way	KAUEN005-20	*Planococcus lilacinus*
6	Blp1kan	KAUEN006-20	*Planococcus* sp.
7	Par1way	KAUEN007-20	*Antonina graminis*
8	Cyp2way	KAUEN008-20	*Dysmicoccus brevipes*
9	Car1idu	KAUEN009-20	*Xenococcus annandalei*
10	Blp1wayant	KAUEN010-20	*Crematogater rogenhoferi*

Fig 3. Barcodes generated for mealybugs and ants.

Table 16. Seasonal variation of root mealybug population on black pepper

Month	**Mean population/ 15 cm root length (No.)**
March-2019	4.33
April-2019	4
May-2019	5
June-2019	17.34
July-2019	28.34
August-2019	63.34
September-2019	71.67
October-2019	56.67
November-2019	27.67
December-2019	42.34
January-2020	15.34
February-2020	6

4.8.1 Correlation with weather parameters

A correlation analysis was done to check the relation between the mealybug population and weather parameters *viz.,* maximum and minimum soil temperature, rainfall, maximum and minimum relative humidity and the number of rainy days.

The correlation between of population of root mealybugs and soil temperature showed a significant negative correlation with maximum soil temperature with correlation coefficient -0.83. A significant positive correlation was observed with maximum and minimum relative humidity and number of rainy days (0.68, 0.71 and 0.64 respectively). There was no significant correlation observed with minimum soil temperature and rainfall, with correlation values of -0.54 and 0.55, respectively (Table 17).

Table 17. Correlation coefficients of soil and weather parameters with root mealybug population

Weather parameter	**Correlation coefficient**
Maximum soil temperature	-0.83**
Minimum soil temperature	-0.54
Soil moisture	0.61*
Rainfall	0.55
Maximum relative humidity	0.68*
Minimum relative humidity	0.71**
Rainy days	0.64*

** Correlation is significant at P= 0.01 level, * Correlation is significant at P= 0.05 level

4.9 GEOGRAPHICAL DISTRIBUTION OF THE MEALYBUGS

The root mealybug, *F. polysperes* was recorded as the dominant species and was found to be present at seven locations comprising of three districts *viz.,* Kasaragod, Kannur and Wayanad. The mealybug species *F. lingnani*, *F. mangiferacola*, *P. lilacinus* and *A. graminis* were found to be present only at single locations of Wayanad district. The mealybug *Planococcus* sp. was present on the roots of black pepper in Kannur and also on the roots of durian from Wayanad. *D. brevipes* was reported from Wayanad and Thrissur districts while *X. annandalei* was reported only from Idukki district of Kerala. The study recorded maximum root mealybug diversity in Wayanad district.

DISCUSSION

5. DISCUSSION

The results of the study entitled 'Diversity of Root mealybugs of Kerala' is discussed comprehensively using the available literature. Correct identification of pest species is important to understand the species diversity and also to develop effective management strategies.

5.1 SURVEY AND COLLECTION OF ROOT MEALYBUGS AND ASSOCIATED ANTS

A purposive sampling survey was carried out at different locations of Kerala to study the presence of root mealybugs and to document root mealybugs and associated ants. Root mealybugs were observed in 14 locations out of the 30 locations surveyed. Among the different districts, maximum distribution of root mealybug was observed in Wayanad having more locations with mealybug infestations. This confirmed with the findings of (Smitha, 2007; Devasahayam *et al*., 2009 and Ummer, 2016). In Kasaragod, root mealybug populations were observed at three locations namely Parappa, Chaturakinar and Periye. The presence of root mealybugs was not recorded in these district in the earlier studies. This indicate rapid spread of the root mealybugs to newer areas. The devastation of the entire banana orchard and black pepper plantation due to root mealybug in Kasaragod was recorded in the present study.

5.2 TAXONOMIC IDENTIFICATION OF ROOT MEALYBUGS

Taxonomic studies revealed the occurrence of eight mealybug species associated with different host plants. Three species of hypogaeic mealybugs belonging to the genus *Formicococcus* Takahashi *viz*., *F. polysperes, F. lingnani* and *F. mangiferacola* were recorded in the present study. Among these *F. polysperes* was recorded from the roots of black pepper, banana, avocado, and the common weed, goat weed. The infestation of black

pepper root with *F. polysperes* in Kerala was also reported by Williams, (2004); Devasahayam *et al*., (2009) and Ummer (2016). The common weed, nut grass was seen harbouring root mealybug, *F. lingnani*. This was previously reported from the same host plant from Karnataka (Williams, 2004; Joshi *et al.*, 2020). The occurrence of *F. lingnani* infesting nut grass roots from Kerala was first reported in this study. The subterranean mealybug *F. mangiferacola* was seen infesting the roots of mango seedlings from Wayanad. Earlier studies conducted by Williams, (2004) and Nagalakshmi (2019) reported the occurrence of this mealybug from Maharashtra and Karnataka.

Two root mealybug species belonging to *Planococcus* were recorded in the study. The coffee roots were infested with *P. lilacinus* and black pepper and durian roots were seen infested with *Planococcus* sp. Pineapple and the weed nut grass were seen to be infested with *Dysmicoccus brevipes*. Similar findings were reported by Bendov, (1994); Williams (2004) and Ummer (2016) where they reported the presence of *D. brevipes* from various host plants in India. The present study reported the incidence of *Antonina graminis* infesting roots of para grass from Kerala for the first time. Previously it was seen infesting sugarcane roots as reported by Pruthi and Rao (1942) from Coimbatore and New Delhi as cited by Williams (2004). Subterranean mealybug, *Xenococcus annandalei* was seen inhabiting the roots of cardamom. This mealybug was first reported and described from Barkuda island, Orissa, India by Silvestri, (1924) and later from Kerala by Deepthy *et al*., (2017).

5.3 DIVERSITY AND HOST RANGE OF ROOT MEALYBUGS

The study identified eight species of mealybugs belonging to five genera associated with 11 host plants from different parts of Kerala. Three species of *Formicococcus* Takahashi *viz., F. Polysperes* Williams, *F. Lingnani* Ferris, *F. mangiferacola* Williams; and two species of *Planococcus* Ferris *viz*., *P. lilacinus* Cockerell, *Planococcus* sp. Ferris and one species of *Antonina* Signoret *viz., Antonina gramins* Maskell; one species of *Dysmicoccus* Ferris *viz., Dysmicoccus brevipes* Cockerell; one species of *Xenococcus* Silvestri *viz., Xenococcus annandalei* Silvestri were recorded in this study. In the first phase

of the study root mealybugs, collected from various host plants from different locations were identified based on morphological characters.

In the present study, the mealybug *F. polysperes* was observed as the predominant root mealybug of crop plants. It was recorded on the roots of black pepper, banana, avocado and goat weed (*Ageratum conyzoides)* from seven different locations. Banana and avocado were the new hosts recorded for *F. polysperes*. Similar findings were reported by Williams (2004) where *F. polysperes* had wider host range. The occurrence of the mealybug on roots of *Piper nigrum* L. (Kerala), *Piper betle* L. (West bengal, Madhya Pradesh and Uttar pradesh), *Arachis hypogaea* L. (Orissa), and *Areca catechu* L. (Uttar pradesh). Later it was also reported on *Curcuma longa* L., *Zingiber officinale* Rosc., *Colocasia esculenta* L., *Amorphophalus paeoniifolius* Dennst., *Ageratum conizoides* L., *Clerodendron infortunatum* L., *Cyperus kyllinga* L., *Phyllantus niruri* L., *Physalis minima* L., *Synedrella nodiflora* L., *Urtica parviflora* Roxb., *Erythrina* sp. from India indicating the invasive nature of *F. polysperes* (Firake *et al*.,2015. & 2018; Ummer, 2016 and Halder *et al*., 2020).

The study found that mealybug *F. lingnani* infesting the roots of nut grass (*Cyperus rotundus* L.) from Pulpally region of Wayanad district and was recorded for the first time in Kerala. The mealybug was previously recorded in India on *C. rotundus* and *A. catechu* from Karnataka (Williams, 2004; Joshi *et al.*, 2020).

The present study revealed the presence of *F. mangiferacola* on the roots of mango seedlings from Ambalavayal region of Wayanad for the first time in Kerala. It was earlier recorded on the roots of mango from the states of Maharashtra and Karnataka of India (Williams, 2004; Nagalakshmi, 2019).

The research findings of the study showed the presence of *D. brevipes* on the roots of pineapple and nut grass from Vellanikkara and Meenangadi of Kerala respectively. This mealybug species has a cosmopolitan pest status and commomly known as pineapple mealybug and has invasive potential and was known to attack all the plant parts of pineapple including the roots (Beardsley, 1993; Gonzalez – Hernandez *et al*., 1999). It was

also reported infesting the roots of soyabean, ground nuts (Thippaiah and Kumar, 1999; Huang *et al*., 2002). Incidence of of *D. brevipes* was also reported on the roots of plants belonging to Poaceae family from Orissa (Williams 2004). Infestation of *D. brevipes* on the roots of black pepper from Kerala was reported by Devasahayam *et al*., (2009) and Ummer, (2016).

The present study discovered the presence of mealybug *P. lilacinus* on roots and collar region of coffee. This aligns with the earlier findings of Sekhar (1964) as cited by Williams (2004) and Koya *et al*., (1996) where he reported the incidence of *P. lilacinus* on the roots and basal stem region of coffee. The occurrence of *Planococcus* sp. on the roots and collar region of durian and black pepper from Wayanad and Kannur districts were also recorded in the present study. Root mealybugs, *Planococcus* sp. and *P. lilacinus* along with three other mealybug species were reported to colonize the roots and basal stem region of black pepper (Devasahayam *et al.,* 2009). Sirisena *et al*. (2013) reported the infestation of durian plants by mealybug *P. citri* from Sri Lanka.

In this study, the mealybug infesting roots of para grass was identified as *A. gramins* and this mealybug was earlier recorded feeding on the roots of sugar cane from New Delhi and Coimbatore by Pruthi and Rao (1942) as cited by Williams (2004).

Xenococcus annandalei was recorded on the roots of cardamom in the present study and similar findings were recorded by Deepthy *et al*., (2017) wherein the infestation was observed on the roots of cardamom and other crop plants and weeds from different localities of Idukki.

5.4 ANT ASSOCIATION WITH ROOT MEALYBUGS

The results of the current study show that the mealybugs associated with different ant species from various locations studied. The mealybug *F. polysperes* is associated with four ant species *viz. ,N. indica, T. indicum, M. brunnea and C. rogenhoferi* from Kasaragod, Kannur and Wayanad districts. Whereas the other mealybugs reported in the study *viz., F. lingnani, D. brevipes, Planococcus* sp., and *X. annandalei* were attended by ant species *N.*

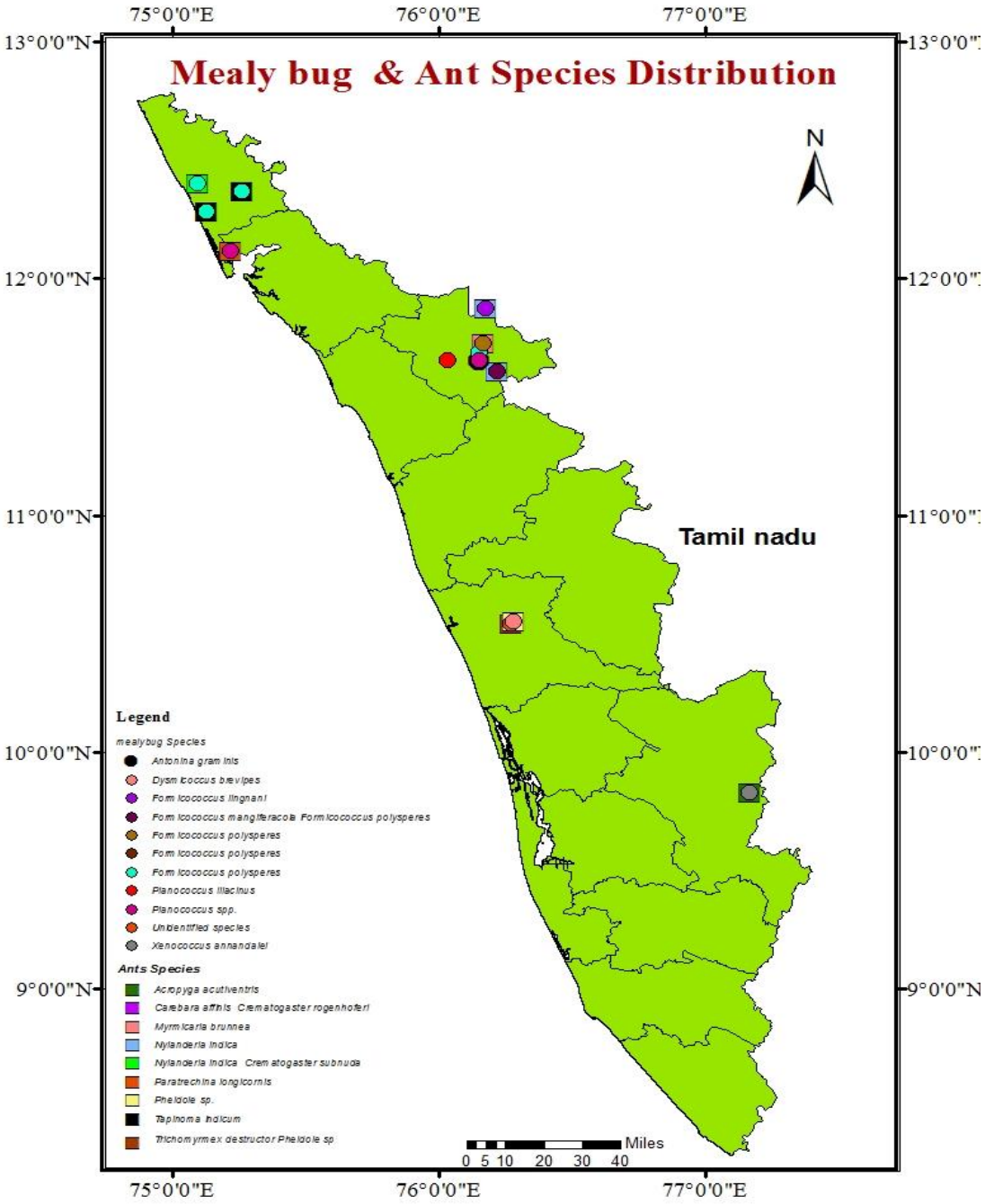
indica, C. affinis, P. longicornis and *A. acutiventris* respectively (Fig 4). Similar results were obtained by Venkataramaiah and Rehman (1989) where they reported nine species of ants associated with coffee mealybug from Coorg and Wayanad regions. The ants were identified as *Crematogater* sp., *Tapinoma melanocephalum*, *Anaplolepis longipes*, *Oecophylla smaragdina*, *M. brunnea, Technomyrmex albipes, P. longicornis, Acropyga* sp. and *Plagiolepis* sp. Studies by Devasahayam *et al.,* (2009) also recorded the presence of five ant species out of which three were identified as *Anoplolepis* sp., *Crematogaster* sp., and *Technomyrmex* sp. along with two unidentified ant species attending the root mealybugs in infested black pepper plantations. Ummer (2016) also reported the occurrence of four species of ants *viz., Anoplolepis gracilipes* Smith, *C. rogenhoferi* Mayr, *Lophomyrmex quadrispinosus* Jerdon and *Paratrechina* sp. associated with root mealybugs in pepper plantations of Kerala. Deepthy *et al*., (2017) reported that root mealybug *X. annadalei* was associated with ant species *A.acutiventris* which helped in the distribution and rapid spread of the mealybugs. Swathi (2018) during her study found that pepper root mealybugs were associated with ant species *P. longicornis, Plagiolepis* sp. and *Aphaenogaster* sp. from Karnataka.

5.5 MOLECULAR CHARACTERISATION

5.5.1 Sequence homology analysis of mealybugs and ants

The homology of the sequences generated in the study with that of sequences already deposited in NCBI was undertaken. The sequences generated for morphologically identified specimens of mealybugs and ants *viz., F. polysperes*, *P. lilacinus*, *D. brevipes* and *C. rogenhoferi* showed significant homology with the corresponding sequences. Whereas the sequences generated for the species *viz., F. mangiferacola, F. lingnani, A. graminis* and *X. annandalei* showed similarity ranging from 83.37 to 94.06 per cent with the sequences present in the database, but that did not match with the morphological identity. Since there is no

Fig 4. Geographical distribution of mealybugs and ants



sequence data of this specific primer available for these mealybugs in the database, significant sequence similarity was not obtained. The sequences of these species were deposited in the NCBI database for the first time in the present study.

The sequences of *F. mangiferacola* and *F. lingnani* showed similarity with that of *P. vovae* (JF714163.1) this is in consonance with the findings of Nagalakshmi, (2019) where *F. mangiferacola* showed similarity with *Planococcus* sp. with both mtCO1 and ITS2 locus. The sequences generated for the mealybug *A. graminis* and X. *annandalei* showed only 91 and 83.37 per cent similarity with *D. brevipes* with NCBI accession LC121504.1 and LC121505.1, respectively. Sequence data of *A. graminis* and *X. annadalei* were not available with NCBI database, hence no significant similarity observed. From this study, sequence data of these root mealybugs were deposited to NCBI database.

5.5.2 Phylogenetic analysis

The sequences generated for the mealybugs in the present study were studied for phylogenetic relationship by constructing maximum parsimony tree and the mealybug sequences of *F. polysperes*, *P. lilacinus* and *D. brevipes* formed a clade with the sequences of corresponding mealybugs retrieved, but the sequences generated for mealybugs *viz., F. mangiferacola, F. lingnani, A. graminis* and *X. annandalei* formed separate clades. The clade formed by *F. mangiferacola* and *F. lingnani* was in between the clades formed by *Planococcus* species, similar results were observed by Nagalakshmi (2019) where *F. mangiferacola* showed similarity with *Planococcus* sp. and in case of *F. lingnani*, this mealybug was first described as *P. lingnani* (Williams, 2004) this could be the reason for the mealybugs *F. mangiferacola* and *F. lingnani* to form clade between *Planococcus* species.

The root mealybug, *A. graminis* formed a separate clade nearer to the clade formed by *D. brevipes*, similar phylogenetic tree was observed in the study conducted by Wang *et al*., (2016) where the clades of *Antonina* sp. was formed near to *Dysmicoccus* sp.

Xenococcus annandalei also formed separate clade and showed divergence from other mealybugs, this could be due to the reason that *X. annandalei* now belongs to a new family Xenococcidae and not under mealybug family Psuedococcidae (Garvrilov- Zimin, 2018 and Hodgson, 2020). According to Garvrilov- Zimin (2018), Xenococcidae showed more similarity with family Margarodidae.

5.5.3. Submission of sequences to BOLD

The study generated ten mtCO1 sequences representing eight species of mealybugs *viz*., *F. polysperes*, *F. lingnani*, *F. mangiferacola*, *Planococcus* sp., *P. lilacinus*, *A. graminis*, *X. annandalei* and one species of ant *viz*., *C. rogenhoferi* and these sequences were submitted to BOLD and illustrative barcodes were generated. In genus *Formicococcus,* the sequences of *F. polysperes* and *F. mangiferacola* had been submitted prior in the database whereas *F. lingnani* was submitted for first time. In genus *Planococcus*, the sequences of *P. lilacinus* and *Planococcus* sp. had been submitted and prior records of these species were present in the database. In case of genus *Dysmicoccus* and *Antonina*, the sequence of *D. brevipes* and *A. graminis* were present in the database and the sequences generated in the study were also submitted. The sequences of mealybug *X. annandalei* was submitted to the database for the first time and the sequence of ant *C. rogenhoferi* was already present in the database and the sequence generated in the current study was successfully deposited in the database.

5.6 SEASONAL INCIDENCE OF ROOT MEALYBUG POPULATION

The study on seasonal incidence of root mealybug *F. polysperes* during the period of March 2019- February 2020 revealed that the population was observed throughout the period of study. The population showed an increasing trend during the rainy season and the maximum population of mealybugs was in September 2019 (71.67/ 15cm root length) followed by August 2019 (63.34/ 15cm root length) and October 2019 (56.67 15cm root length) as the southwest monsoon slowly subsides. The lowest root mealybug population

was recorded during the summer months *i.e.,* March and April 2019 with a population of 4.34 and 4 respectively (Fig 5 to 11).

The results are in agreement with Smitha (2007) in which the population of root mealybug *Geococcus* sp. began to increase with the commencement of the rainy season in June and reached the maximum population in July and also the least population was observed in January to April. Similar findings were also made by Swathi (2018) where the highest population of *F. polysperes* was observed in October followed by September and lowest in March. Findings of Smitha (2007) also were in line with the present study where it was reported that the population of *Geococcus* sp was higher during rainy season (June to September 2005)

However, the results by Ummer (2016) contradicted with the present findings where it was reported that the population of *F. polysperes* was higher during the cooler months of November, December and January and the lowest population was observed during the months of rainy season *viz.,* June and July. The subtropical climate in Wayanad with lower soil temperatures and well-distributed NE monsoon prevailed during the cooler periods *i.e.,* November to January favoured population build-up of root mealybugs. Mean soil temperature of Wayanad recorded from November to January 2015 ranged between 24.83 to 26.80 ^{0}C and the number of rainy days ranged between 2 to 18. Studies on seasonal incidence of root mealybugs were conducted at Kasaragod from 2018 March to 2019 April where soil temperature ranged between 39.65 to 44. 80 and rainy days ranged between 0 to 2.

Fig 5. Population of root mealybug *Formicococcus polysperes* as influenced by maximum soil temperature

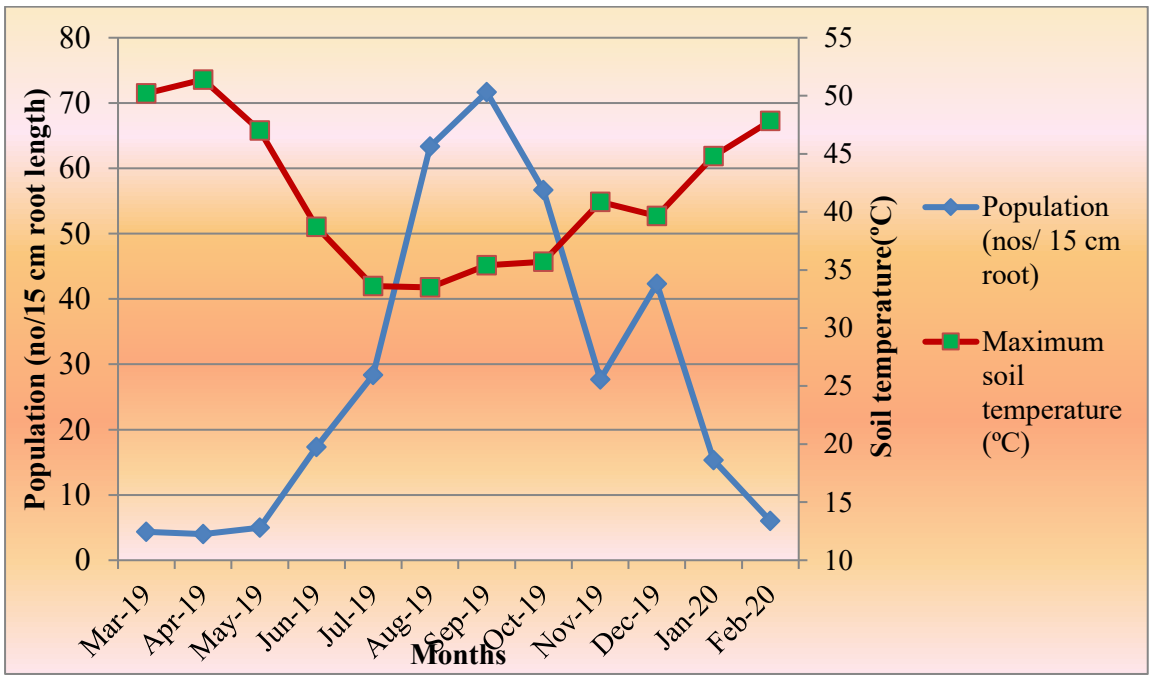


Fig 6. Population of root mealybug *Formicococcus polysperes* as influenced by minimum soil temperature

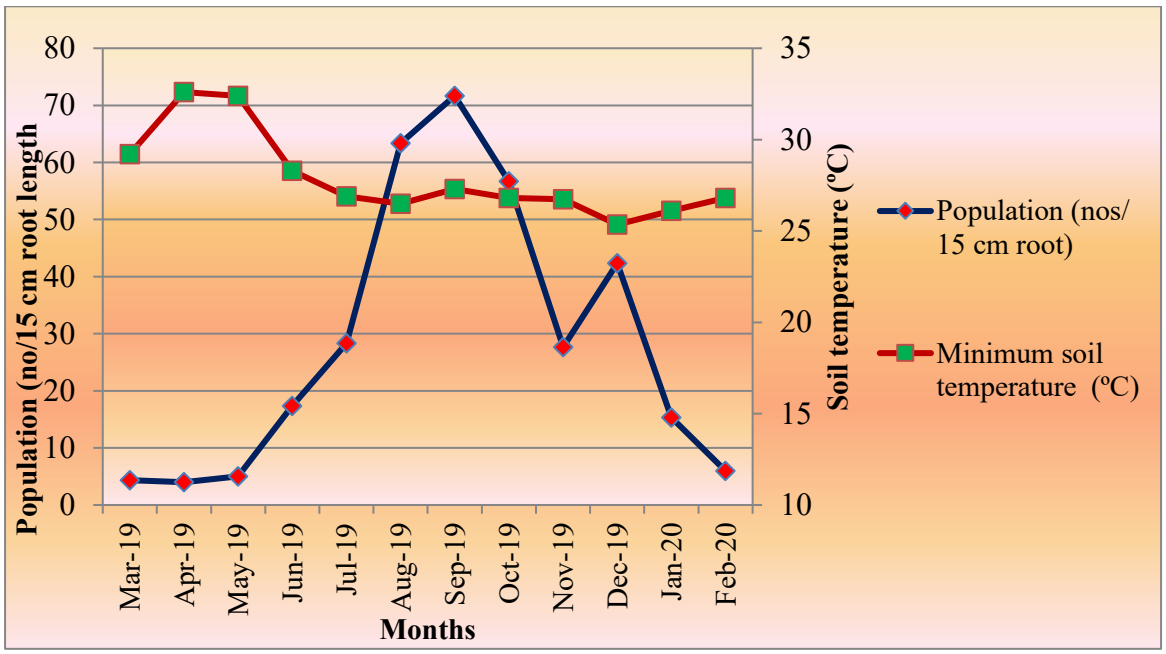


Fig 7. Population of root mealybug *Formicococcus polysperes* as influenced by rainfall

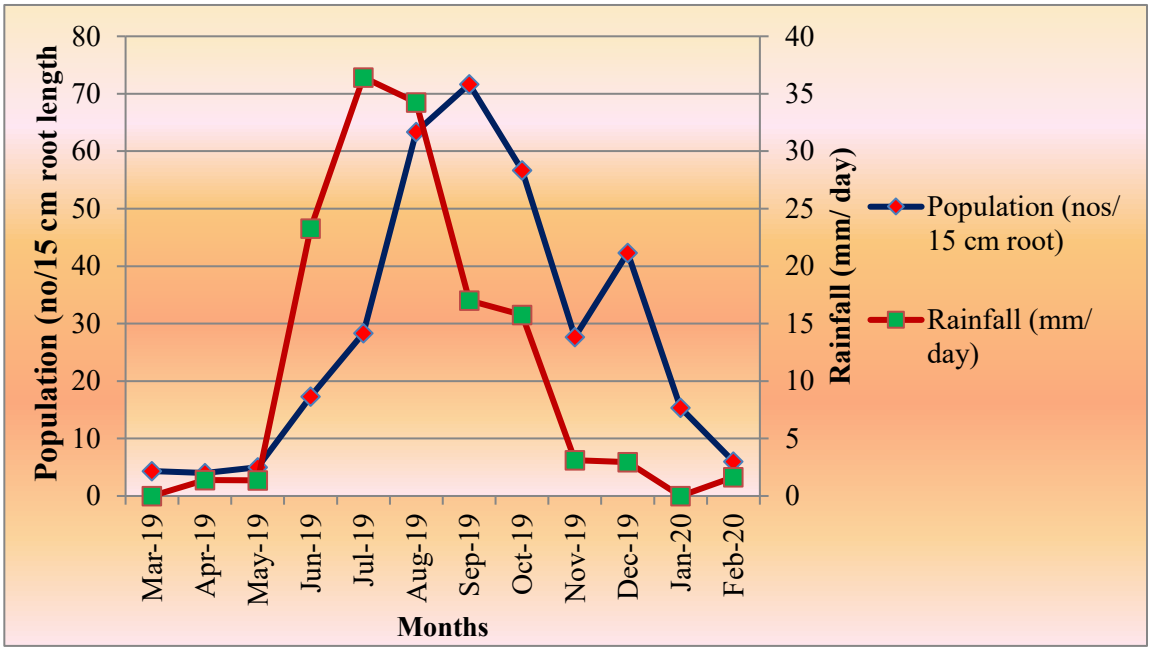


Fig 8. Population of root mealybug *Formicococcus polysperes* as influenced by number of rainy days

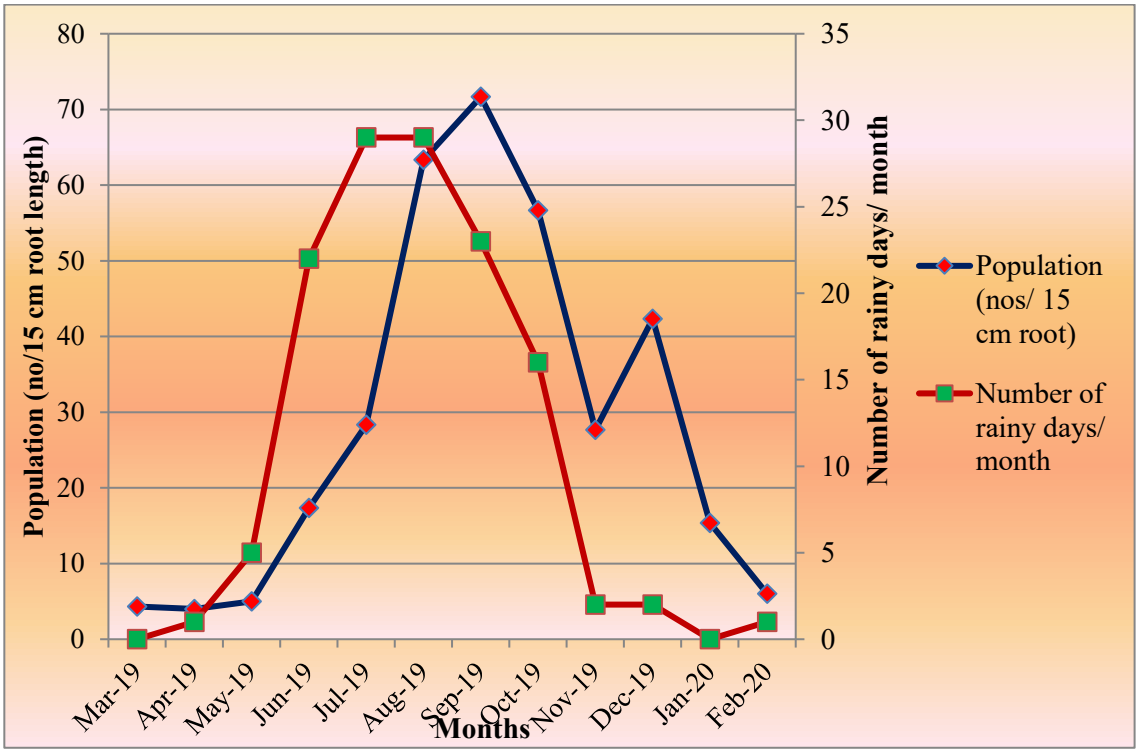


Fig 9. Population of root mealybug *Formicococcus polysperes* as influenced by maximum relative humidity

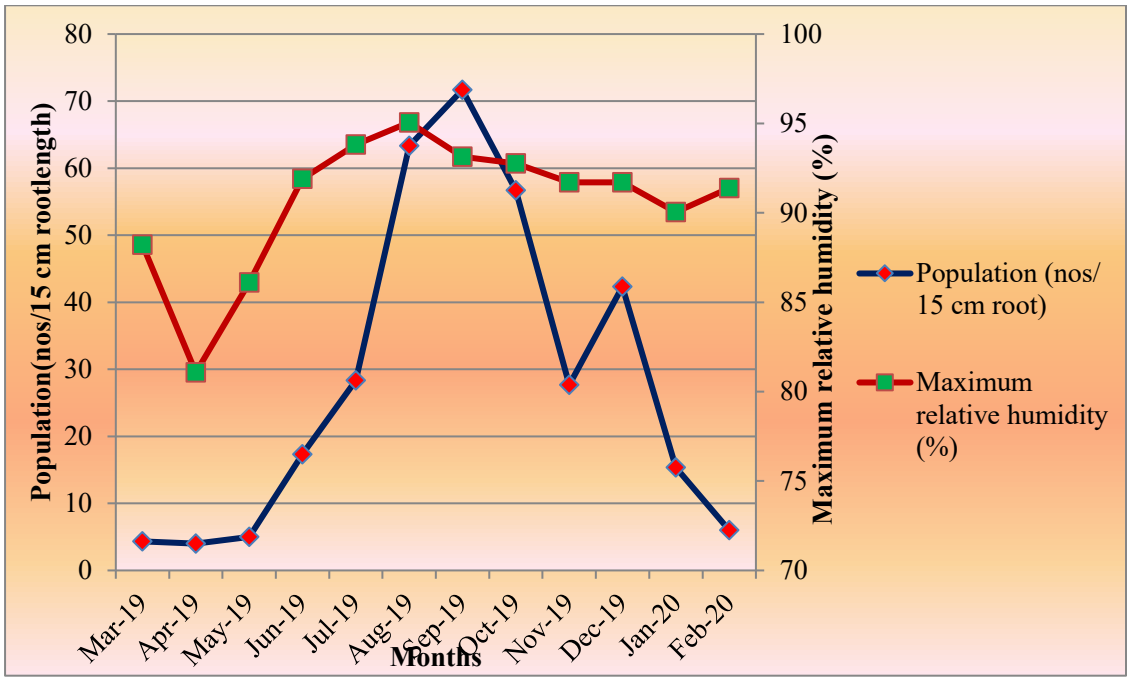


Fig 10. Population of root mealybug *Formicococcus polysperes* as influenced by minimum relative humidity

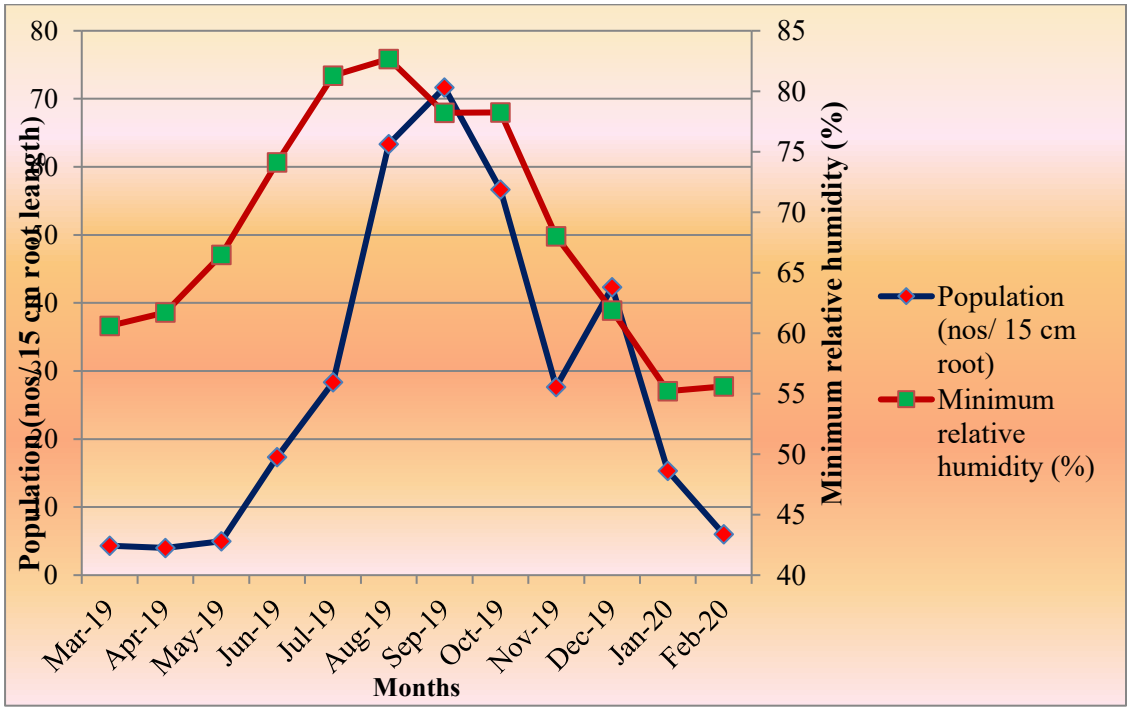
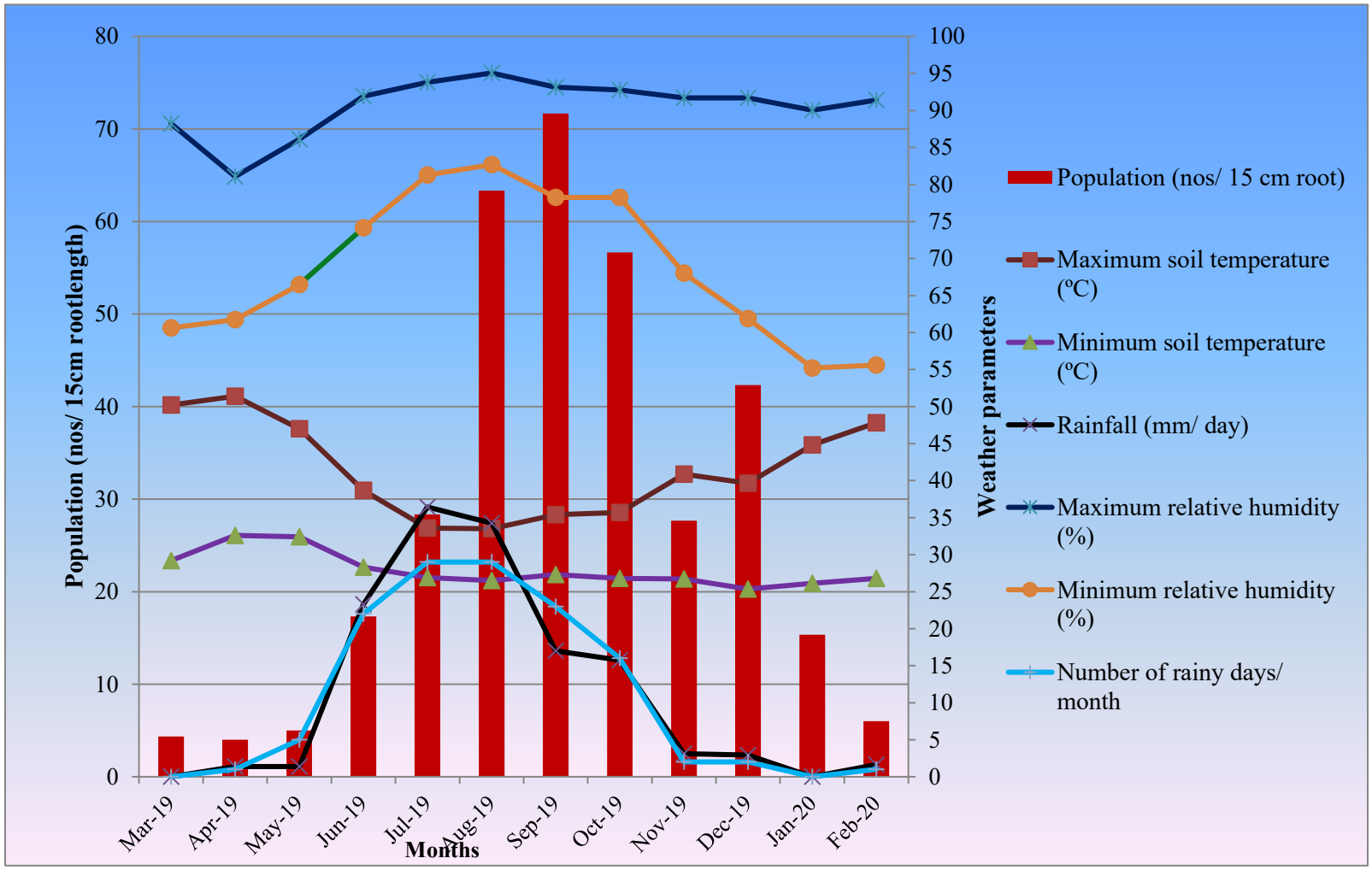


Fig 11. Population of root mealybug *Formicococcus polysperes* as influenced by weather parameters



5.6.1 Correlation with weather parameters

The relationship between weather parameters like soil temperature, relative humidity, rainfall and number of rainy days per month with mealybug population was studied by conducting correlation analysis. The results showed that there was a significant negative correlation between maximum soil temperature with a correlation coefficient of -0.83 whereas a significant positive correlation with that of maximum and minimum relative humidity and number of rainy days per month with correlation coefficient 0.68, 0.71 and 0.64 respectively.

In the case of correlation with soil temperature, similar findings were reported by Smitha (2007) in root mealybug *Geococcus* sp. where the population showed a correlation coefficient of -0.70 with that of maximum soil temperature. Sahu *et al.,* (2017) also observed similar results in case of aerial mealybug *Phenacoccus solenopsis* where the correlation with maximum and minimum temperature showed a highly significant negative correlation.

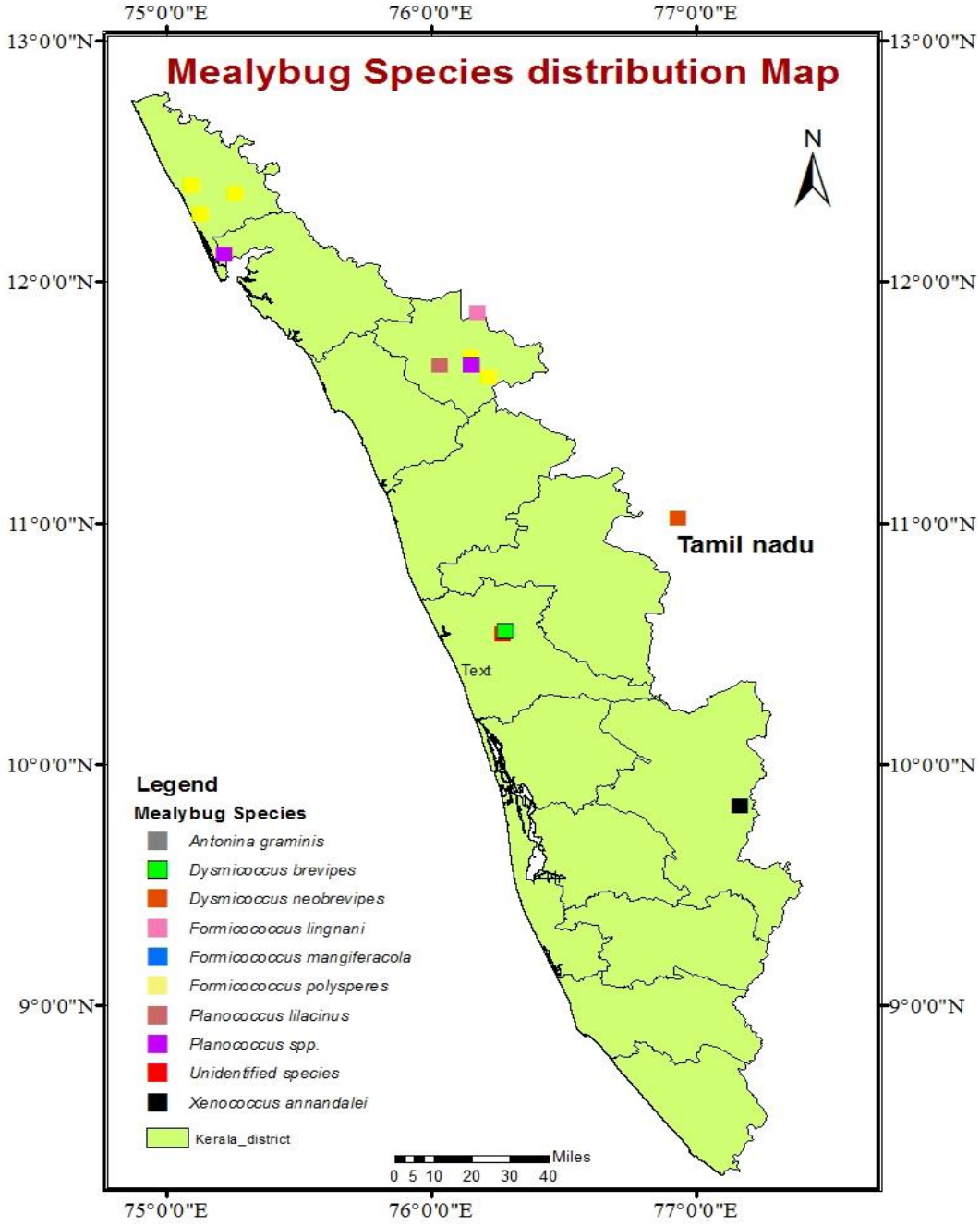
In the case of relative humidity, similar results were observed by Gundappa *et al*., (2018) in mango mealybug *Drosicha mangiferae* where the population showed a significant positive relation with maximum relative humidity and rainfall.

5.7. GEOGRAPHIC DISTRIBUTION

The most dominant root mealybug found in the study was *F. polysperes* which was present at seven locations comprising of three districts of Kerala *viz*., Kasaragod, Kannur and Wayanad (Fig 12) The results of a previous study by Ummer, (2016) indicated that *F. polysperes* was the major root mealybug present in pepper plantations from Wayanad and Idukki but no record of this mealybug from Kannur and Kasaragod districts. Three mealybugs were reported for the first time in Kerala from Wayanad districts *viz*., *F. lingnani*, *F. mangiferacola* and *A. graminis* which were earlier recorded in India (Williams, 2004; Nagalakshmi, 2019; Pruthi and Rao, 1942 as cited by Williams, 2004). *Dysmicoccus brevipes* was recorded from Wayanad and Thrissur district earlier records of the presence

of this root mealybug from Wayanad and Thrissur was reported by Devasahayam *et al*., (2009) and Ummer (2016). The mealybug *X. annandalei* and similar results were observed in the study by Deepthy *et al*., (2017) wherein this root mealybug was observed on a wide range of hosts from Idukki.

Fig 12. Geographical distribution map of mealybugs



SUMMARY

6. SUMMARY

A study on 'Diversity of root mealybugs of Kerala' was carried out at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara to understand the diversity of root mealybugs and associated ants from different crops of Kerala and also to assess the host range, distribution and seasonal incidence of mealybugs infesting underground plant parts. The results of the study are summarized below.

- Purposive sampling surveys were conducted from at 30 different locations of Kerala, covering seven districts of Kerala to collect the root mealybug and associated ants from different crop plants and weeds. The root mealybugs infesting different crops along with ants were collected and stored in ethanol (90 %) from different host plants *viz.,* black pepper, banana, cardamom, pineapple, coffee, avocado, mango, durian, para grass, nut grass and goat weed.
- The morphological identification of the mealybugs revealed that eight species of mealybugs were infesting 11 different host plants. The mealybug *Formicococcus polysperes* was found to be infesting black pepper, banana, avocado and goat weed. Mealybug *Formicococcus lingnani* and *Formicococcus mangiferacola* were identified to be infesting nut grass and mango respectively. The mealybugs on the coffee roots were identified as *Planococcus lilacinus*. *Planococcus* sp. was found to be associated with the roots of black pepper and durian. the mealybug collected from the roots of pineapple and nutgrass was identified as *Dysmicoccus brevipes*. The mealybug *Antonina graminis* and *Xenococcus annandalei* were collected and identified from the roots of para grass and cardamom respectively. The results indicate that the mealybug *F. polysperes* has widened its host range and as reported on the roots of banana and avocado.
- A total of 17 root mealybugs were collected and among them, the highest diversity was of genus *Formicococcus* Takahashi represented by three species *viz., F. polysperes*, *F. lingnani*, *F. mangiferacola*. The genus *Planococcus* was represented by *P. lilacinus* and

Planococcus sp. The genus *Dysmicoccus*, *Antonina* and *Xenococcus* were represented by one species each *viz., D. brevipes*, *A. graminis* and *X. annandalei.* Among these mealybugs *F. lingnani, F. mangiferacola* and *A. graminis* were reported for the first time in Kerala.

- The results indicated that black pepper and nutgrass were found to be infested with two species of mealybugs each *viz., F. polysperes* and *Planococcus* sp. with black pepper and *F. lingnani* and *D. brevipes* with nut grass. The other host plants were found to be infested only with single species of mealybug.
- The ants associated with root mealybugs were morphologically identified. The morphological identification revealed that seven species of ants to be associated with different species of mealybugs. The mealybug *F. polysperes* was associated with four ant species *viz., Tapinoma indicum* Forel, *Nylanderia indica* Forel, *Myrmicaria brunnea* Saunders, W.W and *Crematogaster rogenhoferi* Mayr from Kasaragod, Kannur and Wayanad districts of Kerala. The species, *F. lingnani* was associated with ant species *N. indica* from Pulpally of Wayanad district. The species, *D. brevipes* was associated with ant species *C. affinis* from Thrissur district. The mealybug, *Planococcus* sp. on black pepper was associated with ant species *P. longicornis* from Kannur district. The mealybug, *X. annandalei* was associated with *A. acutiventris* from Idukki district.
- DNA isolation could be successfully carried out by using one mealybug specimen and following CTAB method of DNA isolation resulted in good quality of DNA for both mealybug and ant specimens
- Amplification of mtCO1 locus of mealybugs by PCR amplification using primers specified for mealybugs was successfully carried out, whereas amplification of mtCO1 locus of ants could be carried out successfully using universal primers.
- Homology of sequences of mealybugs and ants with that of NCBI database resulted in 95 – 100 percent similarity with the corresponding species for sequences of five species. Whereas for four species *viz., F. mangiferacola, F. lingnani, A. graminis* and *X. annandalei* there was no significant similarity of sequences with that of other mealybug

sequences in NCBI database as the sequences of these species were deposited for the first time.

- 23 mtCO1 sequences were used to construct a phylogenetic tree which had nine sequences generated in the study representing eight species of mealybugs and 13 retrieved sequences representing six mealybug sequences and one sequence of *Coccus viridis* used as an outgroup.
- The phylogenetic tree of mtCO1 sequences showed that *F. lingnani* and *F. mangiferacola* formed clade between the clades of *Planococcus* species representing closer association with the genus. The species *A. graminis* formed a separate clade since there were no sequences available in the database for this species to show homology. The species *X. annadalei* also formed a separate clade and showed divergence, this can because recently this species was transferred into a separate family Xenococcidae.
- Ten mtCO1 sequences generated in the study representing eight species of mealybugs and one species of ant were submitted to BOLD for barcode generation.
- The seasonal incidence of root mealybugs was studied at an farmer's pepper field infested with root mealybug identified as *F. polysperes* for the period of one year *i.e*., March 2019- February 2020 at Balal grama panchayath of Kasaragod district. The study showed that the population of root mealybugs starts to increase during the rainy season and peak population was observed in the month of September (71.67mealybugs/15 cm root) *i.e.,* when the rains subside. The population starts decreasing from October and reaches to the lowest population in the hotter and drier months of March and April (4.3 and 4 mealybugs/15 cm root respectively).
- The correlation studies of the root mealybug population with weather parameters indicated that there exists a significant negative correlation with maximum soil temperature with a correlation coefficient of -0.83. The correlation with maximum and minimum relative humidity and number of rainy days per month was found to be having significance and with a correlation coefficient of 0.68, 0.71and 0.64 respectively. There was no significant correlation with other weather parameters.

- The mealybug *F. polysperes* was recorded from seven locations comprising of three districts *viz.,* Kasaragod, Kannur and Wayanad. The mealybugs *F. lingnani, F. mangiferacola, P. lilacinus, A. graminis* were only observed in Wayanad. *Planococcus* sp. was observed from two districts *viz*., Kannur and Wayanad also the mealybug *D. brevipes* was recorded from Wayanad and Thrissur. But, the mealybug *X. annandalei* was present only in Idukki. From the present study, it is also clear that the maximum diversity of root mealybugs is present in the Wayanad district of Kerala.

I

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REFERENCES

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ANNEXURES

Annexure I

Details of areas surveyed along with locations, GPS co-ordinates and host plants

Sl. no	District	Location	GPS co-ordinates	Host plants
1.	Kasaragod	Parappa	12.3676340N, 75.2626710E	Black pepper (*Piper nigrum*)
2.		Chaturakinar	12.2862880N, 75.1263010E	Banana (*Musa* sp.)
3.		Periye	12.4043770N, 75.0956070E	Black pepper (*Piper nigrum*)
4.		Hosdurg	12.3106390N, 75.0948760E	-
5.		Pilicode	12.2054430N, 75.1627450E	-
6.		Chittarikal	12.3333320N, 75.3811120E	-
7.		Vellarikund	12.3586416N, 75.2892449E	-
8.	Kannur	Cherupuzha	12.2700010N, 75.3707310E	-
9.		Panniyoor	12.0832722N,75.3971791E	Black pepper (*Piper nigrum*)
10.		Pananpuzha	12.1261600N, 75.3617880E	-
11.		Pilathara	12.0704660N, 75.2632810E	Black pepper (*Piper nigrum*)
12.	Wayanad	Meenangadi	11.6550770N, 76.1484820E	Nut grass (*Cyperus rotunus*) Para grass (*Brachiaria mutica*) Durian *(Durio zibethinus)*
13.		Kenichira	11.7256450N,76.1640940E	Avocado (*Persea americana*)
14.		Ambalavayal	11.6055910N, 76.2184270E	Avocado (*Persea americana*) Mango (*Mangifera indica*)
15.		Kottathara	11.6588490N,76.0306219E	Coffee (*Coffea canephora*)
16.		Kambalakkad	11.6908440N, 76.0794220E	Black pepper (*Piper nigrum*) Goat weed (*Ageratum conyzoides*)
17.		Kalpetta	11.6423425N,76.0888239E	-
18.		Pulpally	11.8653284N,76.1735954E	Nut grass

				(*Cyperus rotunus*)
19.	Thrissur	Vellanikkara	10.5498490N, 76.2775400E	-
20.		Vellanikkara	10.5498490N,76.277540E	Pineapple (*Ananas comosus*)
21.		Thalikund	10.3041730N, 76.183030E	-
22.		Nadathara	10.5098782N, 76.2739204E	-
23.		Kannara	10.5373702N, 76.3204075E	-
24.	Ernakulam	Vazhakulam	9.9437540N, 76.6366671E	-
25.	Kottayam	Kuruvilangad	9.7585780N, 76.563515E	-
26.	Idukki	Thannimood	9.8273960N, 77.1629160E	Cardamom (*Elettaria cardamomum*)
27.		Puttapady	9.7021090N, 77.1882360E	-
28.		Pampadumpara	9.7990340N, 77.1613540E	-
29		Puliyanmala	9.7508902N, 77.144635E	-
30.		Todupuzha	9.8744210N, 76.7028050E	-

Annexure II

Physiochemical properties of soil

Sl.no	Location	Soil pH	Soil moisture
1.	Parappa	5.2	5.3
2.	Chaturakkinar	5.5	4.21
3.	Periye	5.3	11
4.	Panniyoor	5.3	13.12
5.	Pilathara	5.9	17.63
6.	Meenangadi	6.2	7.21
7.	Kenichira	5.8	19.32
8.	Ambalavayal	6.1	12.41
9.	Kottathara	5.2	13.23
10.	Kambalakkad	5.1	18.16
11.	Pulpally	7.4	16.23
12.	Vellanikkara	5.1	5.83
13.	Vellanikkara	5.4	14.61
14.	Thanimood	5.7	14.22

Annexure III

Composition of CTAB buffer (100 ml)

1M Tris HCl (pH 8) – 10 ml (1.576 g/ 10 ml)

5M NaCl - 30 ml (8.775 g/ 30 ml)

0.5 M EDTA (pH 8) - 2 ml (1.8612 g/10 ml)

2 % CTAB – 2 g (2 g/ 100ml)

Distilled water - 54 ml

Annexure IV

Details of weather parameters

Months	Soil temperature		Rainfall (mm/day)	Relative humidity		Number of rainy days/ month
	Minimum (ºC)	Maximum (ºC)		Minimum (%)	Maximum (%)	
March 2019	29.2	50.2	0	60.61	88.22	0
April 2019	32.6	51.4	1.39	61.73	81.07	1
May 2019	32.4	47	1.36	66.48	86.1	5
June 2019	28.3	38.7	23.27	74.13	91.9	22
July 2019	26.9	33.6	36.43	81.29	93.81	29
August 2019	26.5	33.5	34.25	82.68	95.06	29
September 2019	27.3	35.4	17	78.23	93.13	23
October 2019	26.8	35.7	15.75	78.26	92.77	16
November 2019	26.73	40.87	3.12	68	91.71	2
December 2019	25.35	39.65	2.94	61.87	91.71	2
January 2020	26.11	44.8	0	55.21	90.04	0
February 2020	26.8	47.82	1.63	55.61	91.39	1

ABSTRACT

Diversity of root mealybugs of Kerala

By

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ABSTRACT OF THE THESIS

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Diversity of root mealybugs of Kerala

Abstract

Mealybugs (Homoptera: Pseudococcidae) are oval, soft bodied insects causing severe damage to plants not only by sucking the sap but also as vectors of viral diseases of crop plants. The mealybugs infesting roots of crop plants are termed as subterranean mealybugs or root mealybugs. Honeydew excreted by mealybugs attracts ants, and in turn they provide protection to mealybugs from their natural enemies and also help in transportation to other fields. Recently, root mealybug infestation was reported from different localities of Kerala. However, studies on diversity of root mealybug - ant association and seasonal incidence were scanty. Hence the present study, 'Diversity of root mealybugs of Kerala' had been carried out to identify the root mealybugs and associated ant species and to study the seasonal incidence, host range and geographical distribution of major root mealybugs.

Purposive sampling surveys were carried out at different districts of Kerala covering 30 locations and 11 host plants. The root mealybugs and associated ants were collected separately in vials with 90 per cent ethanol. The specimens were labeled with sample codes and preserved as per standard protocols.

The study revealed the presence of eight species of root mealybugs belonging to five genera *viz*., *Formicococcus* Takahashi*, Planococcus* Ferris *, Dysmicoccus* Ferris*, Antonina* Signoret and *Xenococcus* Silvestri. Maximum diversity was found in the genus *Formicococcus,* representing three species *viz*., *Formicococcus polysperes* Williams, *Formicococcus lingnani* Ferris and *Formicococcus mangiferacola* Williams. The genus *Planococcus* was represented by *Planococcus lilacinus* and *Planococcus* sp. The genus *Dysmicoccus*, *Antonina* and *Xenococcus* were represented *viz*., *Dysmicoccus brevipes* Cockerell, *Antonina graminis* Maskell and *Xenococcus annandalei* Silvestri, respectively. Three species *viz., F. lingnani*, *F.mangiferacola* and *A. graminis* were recorded for the first time from Kerala.

The root mealybug, *F.polysperes* was recorded to be having wider host range and was found infesting black pepper, banana, avocado and goat weed. Pineapple mealybug, *D. brevipes* was recorded on pineapple and nut grass, while *Planococcus sp*. was reported on durian and black pepper. Root mealybugs, *F. lingnani*, *F. mangiferacola, A. graminis*, *P. lilacinus* and *X. annandalei* were recorded from single host plant each *viz*., nut grass, mango, paragrass, coffee and cardamom respectively.

The morphological identification of ants associated with root mealybugs revealed seven species of ants of which maximum number of ant species were associated with root mealybug, *F. polysperes*. The ants associated with *F. polysperes* were *Nylandria indica* Forel, *Tapionoma indicum* Forel, *Myrmicaria brunnea* Saunders, W.W. and *Crematogaster rogenhoferi* Mayr . The ant associated with *F. lingnani* was *N. indica*; while *Carebara affinis* Emery with *D. brevipes* and *Paratrechina longicornis* Latreille with *Planococcus* sp. on pepper. Ant seen in association with the subterranean mealybug, *X. annandalei* was *Acropyga acutiventris* Roger. Among the ant species, *N. indica* was found to be the major species associated with *Formicococcus* from Kasaragod, Kannur, and Wayanad districts.

The major root mealybug species, *F. polysperes* was, noted from seven locations of three districts *viz*., Kasaragod, Kannur and Wayanad respectively. The root mealybugs, *F. lingnani, F. mangiferacola*, *A. graminis*, *P. lilacinus* and *Planococcus* sp were reported from Wayanad whereas *D. brevipes* was recorded from Wayanad and Thrissur districts.

Molecular characterization of root mealybugs and ants was performed by isolation of genomic DNA and amplification of *mitochondrial cytochrome oxidase one* (mtCO1) locus using specific primers. The sequencing of polymerase chain reaction (PCR) product and *in- silico* analysis of eight species of mealybugs and one species of ant was carried out. The sequence data of the following four root mealybugs *viz*., *F. mangiferacola*, *F. lingnani*, *A. graminis and X. annandalei* were absent in the NCBI data base. The sequences of these mealybugs were deposited in the NCBI database for the first time. Remaining four species of mealybugs showed 91-100 per cent similarity and were in

agreement with the morphological identification. The sequences were submitted to NCBI-Genbank for future access and use. The sequences were also uploaded to Barcode of Life Data systems (BOLD) and generated barcodes for eight species of mealybugs and one species of ant.

The study on seasonal incidence of root mealybug *F. polysperes* showed that the population of mealybugs increased during rainy season from June and reached its peak in the month of September when the rains subside. The population was least during the summer months. Correlation of mealybug population with weather parameters indicated a significant negative correlation with maximum soil temperature and significant positive correlation with relative humidity and number of rainy days.