V. DISCUSSION

The results of the study on morphological and molecular characterization of mealybugs, documentation of mealybugs and associated natural enemies in and around Bengaluru and certain regions of Karnataka, distribution of *Rastrococcus* spp. in mango growing regions of Karnataka are discussed in this chapter.

Mealybugs are well known sap sucking insect. Some also transmit plant viruses. Their feeding often causes considerable economic loss of horticultural and agricultural crops (Golino *et al*., 2002; Miller and Miller, 2002). These are widely distributed phytophagous insects with wide host ranges. According to Miller *et al*., (2005), 158 species of mealybugs are recognized as pests worldwide. These pests have huge negative impact on a wide range of crops. In the present study an attempt was made to characterize mealybugs which were collected from various regions of Karnataka through intensive survey during 2012-2015.

5.1. Morphological characterization

Morphological characterization was a prerequisite to molecular characterization. The results of morphological studies on the family Pseudococcidae comprising 18 species belonging to 11 genera from different regions of Karnataka are discussed here. These were keyed morphologically, after standard slide preparation mainly using the keys of Williams (2004) at the Division of Germplasm Collection and Characterization in Insect Systematics lab at ICAR-NBAIR.

In the genus *Coccidohystrix*, only one species, *C. insolita* was recorded. This species closely resembles *C. eleusines*. However, the absence of dorsal oral collar tubular ducts in *C. insolita* is a major distinguishable character that separates it from *C. eleusines*.

The genus *Dysmicoccus* comprises more than 110 species. At present many species are assigned to it because they cannot be included either in *Pseudococcus* or in *Trionymus*. Species of *Dysmicoccus* have tarsal digitules that are either all knobbed or disparate (one pair of digitules is knobbed and the other flagellate) but in some species, the digitules are all flagellate. Recent studies of primary endosymbionts nucleotide sequences of the type species *Dysmicoccus* have shown close relation to the type species of the genus *Pseudococcus* (Thao *et al*., 2002). The species *D. neobrevipes* is very closely related to the species *D. brevipes*, but the former can be differentiated from *D. brevipes* by the presence of a short setae medially on the abdominal segment VII, immediately anterior to the anal ring.
The species *Ferrisia virgata* can be differentiated from *F. malvastra*, as the former possesses at least eight multilocular disc pores and many more in a double row on abdominal segment VI. Further *F. virgata* differs from *F. malvastra* in having the collars of the setae associated with each enlarged tubular duct situated within the sclerotized rim but in *F. malvastra*, the collars of these setae are situated just outside the sclerotized rim, not within it.

The genus *Formicococcus* has been extended to include a number of species in southern Asia. In 1956 the authors Ezzat and McConnell erected a genus *Planococcoides* for species with an anal lobe bar, 18 pairs of cerarii among which some cerarii possess more than two cerarian setae on the abdomen and also an anal ring bearing six setae. These characters are almost identical to the genus *Formicococcus* which differs from only one character, i.e. presence of more than six setae on the anal ring. In the present study, the specimen is assigned to the genus *Formicococcus* because the anal ring possessed more than six setae. *Formicococcus mangiferacola* closely resembles *Fo. polysperes* in possessing short dorsal setae and ventral oral collar tubular ducts on the margins of the head and thorax, but differed in the shape and size of the dorsal setae. *Formicococcus mangiferacola* differed from the *Fo. polysperes* in having the dorsal setae with 10-17µm long, with the base of each seta being narrower than a trilocular pore.

The species *M. hirsutus* belongs to the genus *Maconellicoccus* and is a very destructive pest mainly in grape vineyards of Karnataka. The species can be easily recognised by its orange and pink coloration which turns black in 70% alcohol and is similar to *M. ramchensis* and *M. multiporii*, which are other species of southern Asia. *Maconellicoccus hirsutus* differs from *M. ramchensis* and *M. multiporii* in possessing a few oral collar tubular ducts around the dorsal segments and in the size of the oral rim tubular ducts.

The genus *Nipaecoccus* is world-wide in distribution (McKenzie, 1967). The species *N. viridis* was described by Zimmerman (1948) and Ferris (1954) as *N. vastator*, De lotto (1957) as *Trionymus serius* and Williams and Watson (1988) as *N. viridis*. The mealybug *N. viridis* can be easily separated from *N. nipae* by its larger size (body up to 4.0mm long), possessing lanceolate dorsal setae, presence of multilocular disc pores and oral collar tubular ducts in the ventral margin area of thorax.

The genus *Paracoccus* was included in the tribe Planococcini (Ezzat and McConnel, 1956) as it possessed anal lobe bars. It was originally described as possessing 17 pairs of cerarii but later Cox (1987) redefined the genus to include species with 18 pairs of cerarii with the preocular pair (C2) either present or absent. Sometimes a few species of this genus was
difficult to separate, hence some species were assigned to *Pseudococcus*, but the presence or absence of anal lobe bars clearly separated the genera. The papaya mealybug *P. marginatus* can be easily distinguished from other *Paracoccus* species by the presence of oral rim tubular ducts dorsally restricted to marginal areas of the body, absence of translucent pores on hind tibiae and femur (Miller and Miller, 2002) and translucent pores restricted only to coxa.

The genus *Phenacoccus* can be identified by the presence of short lanceolate setae and possessing nine segmented antennae and a denticle or tooth on the claw. Generally the presence of claw with denticle is helpful in defining the members of the genus *Phenacoccus* (McKenzie, 1967). *Phenacoccus solani* is very similar to *Ph. solenopsis* by the lack of quinquelocular pores, dorsal multilocular pores and dorsal oral-collar tubular ducts. However, *Ph. solenopsis* has mushroom shaped circulus and its multilocular disc pores are restricted to abdominal segments VI, VII and VIII. Further, dorsal setae in *Ph. solani* has strikingly different lanceolate shape. Based on Hodgson et al., (2008), morphological variation found in Asian species considered that *Ph. solani* and *Ph. solenopsis* might be environmentally-induced variants of a single species. The species *Ph. divaricatus* can be differentiated from other species of *Phenacoccus* by the presence of diverging tubular ducts. In the past, *Ph. madeirensis* was frequently misidentified as *Ph. gossypi*ii, but *Ph. madeirensis* can be distinguished from *Ph. gossypi*ii in possessing dorsal multilocular disc pores which are rare on the thorax and if present they appear on the margins only. The species *Ph. parvus* is polyphagous but with a host preference for lantana and the members of Solanaceae.

The genus *Planococcus* could be confused with the genus *Paracoccus* because of the presence of oral rim tubular ducts. So this was changed to *Pl. morrisoni* Ezzat and McConnell to *Paracoccus interceptus* Lit (Lit, 1997). Some species belonging to the genus *Planococcus* are difficult to identify. The species *Pl. citri* can be sometimes easily distinguished from most of the other species of *Planococcus* by its venter of head with 14 to 35 oral collar tubular ducts and thorax with total of 7-30 ducts near eighth pair of cerarii (*C₈*) and ventral ducts on head and next to eighth pair of cerarii totalling 15-50. But it is difficult to separate it from *Pl. minor* because of the variation in numbers of ventral oral collar tubular ducts. The variation in the characters of these two species were discussed in detail by Cox (1981, 1983, 1989). The species *Pl. lilacinus* differs from *Pl. citri*, where in the former multilocular disc pores are usually absent from margins and submargins of abdominal segments (occasionally 1 or 2 present), cerarii present with long flagellate setae on the head portion.
The only genus confused with *Pseudococcus* is *Dysmicoccus*. Many species of *Dysmicoccus* possess cerarii similar to *Pseudococcus*, but oral rim tubular ducts are always present in *Pseudococcus* and absent in *Dysmicoccus*. The species *Ps. longispinus* is very closely related to the Pacific species of *Ps. microadonidum* Beardsley and *Ps. marshallensis* Beardsley. But *Ps. longispinus* can be distinguished from both, by the size of the penultimate cerarii, each about 65-75µm in diameter, whereas in the other two species the penultimate cerarii is almost 50µm in diameter. The ventral sclerotized area on each anal lobe in *Ps. longispinus* is conspicuous, where as in other two species it is much smaller.

The genus *Rastrococcus* does not have close relatives except *Lankacoccus*, a genus with similar cerarii but with all the cerarian setae lanceolate instead of truncate-conical. *Rastrococcus* was included in the genera *Phenacoccus* by the presence of quinquelocular pores, 9-segmented antennae, minute lanceolate dorsal setae and tarsal digitules that are flagellate. The species *R. iceryoides* is easily recognisable by distorted trilocular pores, the long flagellate setae flanking anal ring and with two pairs of ostioles. The species *R. invadens* is differentiated from *R. iceryoides* by not having anterior pair of ostioles but only posterior pair of ostioles are present and long flagellate setae are absent in flanking anal ring and only a short lanceolate setae are present in this position. Most of the cerarii are in same size as cerarii situated nearby anal ring. Trilocular pores are present on inner edges of cerarii on head, thorax and few in anterior abdominal segments which are more or less single rows. *Rastrococcus invadens* is very close to *R. spinosus* but differs in possessing few intermediate cerarian setae. *Rastrococcus mangiferae* is easily distinguished from other species of this genus by having multilocular disc pores possessing large loculi and is called as a wagon-wheel type (Williams, 1989). *Rastrococcus mangiferae* is closely related to *R. chinensis* but *R. mangiferae* can be distinguished as it possesses multilocular disc pores on the venter of the thorax. The study clearly showed how morphological characters are primary and crucial in the correct identification of the species.

5.2. Molecular characterization

Mealybugs namely *M. hirsutus*, *Planococcus* spp., *Ps. longispinus* and *N. viridis* are commonly occurring pests of horticultural crops in India (Singh, 2003). *Phenacoccus solenopsis* (Hodgson *et al.*, 2008), *Ph. solani* (Suresh and Kavitha, 2007; Anon, 2013a) and *P. marginatus* (Muniappan *et al.*, 2008) were introduced recently and have now become predominant on many crop plants. Species of *Rastrococcus* have been recorded earlier from India (Narasimham and Chacko, 1991), mainly on *M. indica*, but our recent surveys indicated *Fo. mangiferacola*, as
one of the emerging pest on mango. A total of 15 different mealybug species was clearly differentiated on the basis of the MT-CO1 and ITS2 gene region successfully and is a important tool for identification of these pests (Park et al., 2011). In the present study it was concluded that the molecular approach can complement classical taxonomy. Different mealybug species were characterized using MT-CO1 and ITS2 gene regions and submitted to NCBI- GenBank for future access and use. Among these 15 species, five namely *R. iceryoides*, *R. invadens*, *R. mangiferae*, *N. viridis* and *Fo. mangiferacola* were deposited for the first time to NCBI-Genbank by targeting MT-CO1 gene. Other identified mealybug species using MT-CO1 gene, namely *M. hirsutus*, *Ps. longispinus*, *P. marginatus*, *Pl. lilacinus*, *Ph. solenopsis* and *Ph. solani* showed maximum identity with other available reports in NCBI-GenBank. The obtained sequence of *Pl. citri* with MT-CO1 showed maximum similarity with already existed sequence of *Pl. citri* in NCBI-GenBank. Earlier reports, showed that morphological and molecular identification of the mealybug species *Pl. citri* and *Pl. minor* are often complicated and difficult (Cox and Wetton, 1988; Rung et al., 2008). Reason for this error might be the DNA dataset disagreed with the morphologically based data set. So it remains a question for taxonomist and even molecular biologist. However, more studies are needed to sort out the conflicting information given by the DNA sequence data. The mealybug *Fo. mangiferacola* formed a separate clade between the *Planococcus* species. Genetic variation was observed between *Planococcus* sp. and *Fo. mangiferacola* (6.78% - 8.17%). This species was deposited for the first time to NCBI-Genbank based on morphological data described by Williams (2004). In previous reports *Fo. mangiferacola* species belonged to the genus *Planococcus* (Varshney, 1992; McKenzie, 1967). *Phenacoccus solani* and *Ph. solenopsis* are considered morphologically closely related species (McKenzie, 1967), we were able to resolve this easily by using MT-CO1 gene approach. In this study less nucleotide variation (4.78%) was observed and these are categorized as different species. In case of *Ph. solani* and *Ph. solenopsis*, the obtained molecular data completely supports the morphological data. The obtained mealybug sequences showed that the GC content in the MT-CO1 region is very low (14.97%) and it is lowest as compared to other insect species (33-53%) (Min and Hicke 2007, and Park et al., 2011). The lowest GC content (17-33%) occurs in bacterial species with small genome types, particularly in the insects endosymbionts such as aphids (Anderson and Kurland, 1998; Moran et al., 2008). The lower GC content in mealybugs might be due to habit of feeding only on plant sap, a diet with lowest organic nitrogen content. It explains evolutionary adaptation to less nitrogen. It was also found that less nitrogen is required for AT than GC (Rebijith et al., 2016). In phylogenetic analysis MT-COI sequences were compared with South Korean and Chinese
sequences drawn from NCBI-GenBank, because most of the mealybug sequences were deposited from these regions. In a phylogenetic tree *M. hirsutus*, *Ph. solani* and *Ps. longispinus* formed separate clades from the corresponding mealybug species sequences obtained from South Korean and Chinese species in NCBI-GenBank. This indicates that there might be a chances of slight differences in morphological characters from India and other countries or different biotypes. These results were met with the morphological and molecular studies on different populations of *Pl. ficus* in Tunisian vineyards (Mansour et al., 2012). A total of nine different species were identified using ITS2 gene region namely *F. virgata*, *Fo. mangiferacola*, *M. hirsutus*, *Ph. madeirensis*, *Ph. parvus* *Ph. solenopsis*, *Pl. citri*, *Pl. lilacinus* and *Ps. longispinus*. ITS2 gene region is very helpful, because distinguishing different species based on insertions/deletions which is highly suitable for identification. In a phylogenetic tree, the mealybug species obtained from the present study viz., *Pl. lilacinus*, *Fo. mangiferacola*, *Pl. citri*, *Ps. longispinus*, *M. hirsutus*, *F. virgata*, *Ph. solenopsis*, *Ph. madeirensis* and *Ph. parvus* showed maximum similarity with corresponding species of Egypt, Korea, Brazil and Spain along with distinct clades and well-supported bootstrap values. However, according to Malausa et al., (2010), both MT-COI and ITS2 gene regions are most important and informative for population genetics as well as "Barcoding of life".

5.3. Documentation of mealybugs and natural enemies from major districts of Karnataka

The mealybugs have huge negative impact on a wide range of crops. In the present investigation an effort was made to characterize and document the mealybug species on horticultural crops from Karnataka which is a horticulturally important state in India (Singha et al., 2014). From intensive surveys, a total of 18 species of mealybugs were collected from different regions of Karnataka from 58 host plants. Natural enemies associated with 13 mealybugs species were collected, identified and documented. The host plants included vegetables, fruits, ornamentals, medicinal plants, weeds and also few non horticultural crops which were incidentally collected.

The mealybug *C. insolita* is a serious pest, attacking brinjal in various regions of India including West Bengal (Chaudhary, 1976), Kerala (Gopinathan et al., 1982) and Bihar (Lall et al., 1976). This mealybug was found on tomato, brinjal and on the ornamental crop *Abutilon indicum* mainly in the southern regions of Karnataka. Release of its predator *C. monstrozieri* has given an outstanding control of *C. insolita* in Andhra Pradesh (Tirumala rao and David, 1958). A single larva of *C. monstrozieri* is known to consume about 1100 nymphs
of *C. insolita*. Among these hosts, *C. insolita* species in tomato and brinjal must be included during regular surveillance as these can spread easily northwards to Ranebennur, Haveri, Hubli, Dharwad etc as these are major vegetable production areas especially seeds (Sudha *et al*., 2006). Sometimes, these mealybugs anchor in the sepals of harvested tomato/brinjal. As these vegetables are sent to Kerala and Tamil Nadu spread of this mealybugs to neighbouring states is a potential threat. So care should be taken to screen vegetables before interstate marketing.

*Dysmicoccus neobrevipes* was also known as ‘grey’ form of pineapple mealybug. This species was considered as a species complex with *D. brevipes*. In Hawaii, investigations have been carried out to separate these two species with taxonomic keys (Beardsley, 1993). In the present investigation, the mealybug *D. neobrevipes* was collected on tuberose from Indian Institute of Horticultural Research (rural region of Bengaluru). Tuberose is one of the most important ornamental and flowering plants cultivated for production of long-established flower spikes. These flowers are commonly used for garden displays, interior decoration, as these flowers remains fresh for a long time, extraction of oils for perfumes and cosmetic products etc. In international markets, there is high requirement for tuberose hence holding a very good price. India is gaining momentum day by day in tuberose cultivation due to huge export prospect. IIHR-Bengaluru is producing most of the varieties of tuberoses. This institute is exporting most of the varieties of tuberoses to other parts of the country. In the present investigation the mealybug was reported on tuberose from IIHR is of importance. This can spread rapidly even to other places. Hence care should be taken while exporting the tuberoses by treating the bulbs with appropriate insecticides. This mealybug species can be controlled by the parasitoids *Anagyrus ananatis* Gahan and *Euryrhopus propinquus* Kerrich and the predator *Lobodiplosis pseudococci* Felt (Beardsley, 1993). But in the present studies, these parasitoids were not recorded because collected specimens were very few in numbers. Surveillance of these mealybug species, therefore on bulbous crops, pineapple, etc will help prevent spread and flare up of the mealybug.

It is interesting to note that *F. virgata* due to its high polyphagous nature was found on fruit plants like custard apple, pomegranate, guava, fig, mango, brinjal, cashewnut and also medicinal plants like blue pea (*Clitorea ternatea*) and pongamia in northern and southern regions of Bengaluru. Among these *F. virgata* has known to cause more damage to the crops viz., guava and custard apple. This pest sucks the sap from the plants, leaves and fruits will turns to yellow and shed. Finally this leads to drying of plants. The leaves and fruits covered with honeydew serves as a intermediary for the growth of sooty moulds. The sooty mould
and waxy deposits results reduction in the photosynthesis. Due to this reason, the fruits loose their market value. This mealybug species is very serious in guava and custard apple. Hence the release of natural enemies in the field can suppress the population of this mealybug. Natural enemies *A. advena* and *S. coccivora* efficiently controlled the population of mealybug within 50 days of release in guava fields (Mani *et al.*, 1990). In the present investigation the natural enemies *B. insularis, A. advena, N. regularis* and *S. coccivora* were recorded from the mealybug. *Ferrisia virgata* being a serious polyphagous pest, needs a strong commercial, biological control. Production of these natural enemies has not yet been scaled up to reach farmers, hence biological control of *F. virgata* is more on papers than in practice.

In the present study mealybug *Fo. mangiferacola* was recorded on mango from southern and Malnad region of Karnataka. This mealybug was reported from India on the roots of mango by Williams (2004). In our studies it was found on the root, shoot and fruits. This indicates this pest may attain a major status in future. During our investigation only one natural enemy *Allotropa* sp. was found attacking this mealybug. Further research has to progress in search of natural enemies to control this mealybug.

*Maconellicoccus hirsutus* is the most important mealybug in horticultural crops. It causes loss in grapes especially on Thompson seedless which is exported (Ghose, 1972). As the mealybug spikes in fruiting stage, farmers resort to spray of dichlorvos, which apparently is safe to the predator *C. montrouzieri* (Mani and Krishnamoorthy, 2001). However, severe residues in the fruits, especially of organophosphate compounds led to ban or rejection of grapes. Thus, the mealybug caused severe loss in foreign exchange. Management strategies have not found adequate satisfactory results. Use of predators *viz.*, *C. montrouzieri, Chilocorus nigrita* (F.), *Exoplectra* sp., *Harmania axyridis* (Pallas) and *Tenuisvalvae notata* (Mulsant), parasitoids like *Anagyrus dactylopii* and *Gyranusoidea indica* (Singh, 2003 and Peronti *et al.*, 2016), fish oil rosin soap (Anon, 2007), botanicals (Sunitha *et al.*, 2009), need to be further researched. In Karnataka, the mealybug is widespread in Bengaluru rural, Kolar, Bijapur and Belagavi where grapes are cultivated. If grape area expands, the mealybugs inevitably spread. So care should be taken to see that the planting materials are free of mealybug egg mass to prevent its introduction in vineyard. Verghese (1997) found that egg masses of *M. hirsutus* below the loose bark in the main stem, so he recommended a swab of COC (copper oxychloride) + Carbaryl, after removal of loose bark, which gave effective control of the mealybug.
It is also believed that females *M. hirsutus* migrates to the soil and lays egg masses there. This needs further confirmation. *Maconellicoccus hirsutus* is also known to occur on mulberry, where insecticidal sprays are not possible. As sericulture is important in Karnataka, attempts to encourage *C. montrozieri* supply through private, commercial nurseries should be seriously taken up both by horticultural departments and government agencies. In case of sapota, early detection of *M. hirsutus* on fruits is important. At this stage, brushing off the mealybugs from the hardy fruit using a soft paint brush is sufficient to prevent establishment of *M. hirsutus* on custard apple. It is pertinent to mention that *M. hirsutus* is an excellent laboratory host on pumpkin to culture *C. montrozieri* for mass production (Joshi et al., 2006).

*Nipaecoccus viridis* is widespread throughout tropics and subtropics causing economic losses to various crops. In the present investigation this mealybug was recorded on fruits, vegetables, ornamental and medicinal crops. This mealybug undergoes natural mortality which is induced by unfavourable weather conditions and the presence of natural biological control agents. The release of two predators *C. montrozieri* and *S. coccivora* was recommended to control *N. viridis* population (Joshi et al., 2006). Natural enemies *A. dactylopii* (Howard) and *S. epius* were recorded on *N. viridis* during the study period.

The papaya mealybug *P. marginatus* was found throughout Karnataka and wherever it occurred, the parasitoid *Acerophagus papayae* was also found. No insecticide could control *P. marginatus*, and hence there is importance for biological control (Shylesha et al., 2010). It is interesting that from a bouquet of natural enemies, only *Acerophagus papayae* was found dominantly occurring. It will be a useful study to see the interspecific competition among these natural enemies. This study helped in monitoring the mealybugs across the state.

*Planococcus lilacinus* is an emerging mealybug pest. Its presence on jackfruit and fig is very significant as these two horticultural crops are minor crops becoming major commercial tree crops. Once these crops are established as monocultures, the mealybug *Pl. lilacinus* can establish well typical of mealybugs. So care should be taken from the planting stage itself. Managing mealybugs in nurseries is very important as pepper is also a host (Anon, 2010). Another mealybug *Pl. citri* was found in many districts like south and north Karnataka, Kodagu, Chikkamagaluru etc spread of *Pl. citri* from pepper to other crops should also be prevented. This mealybug was also recorded on coffee. Hence coffee is also a high economic important crop. Hence the mealybug spread should be prevented before it reaches to other crops. One advantage of biological control is availability of an array of natural enemies.
These have to be mass cultured and released in endemic areas. *Planococcus lilacinus* is also a pest on pomegranate where pesticidal presence is high. It is quite possible that the mealybug may acquire resistance. Insecticidal resistance management is therefore an important area of investigation in pomegranate. In citrus, where *P. citri* is also a pest, interspecific competition between *Pl. citri* and *Pl. lilacinus* will be an interesting study, if they co-occur. Further release of common parasitoids for both (Ex. *Leptomastix* sp) will be a time, money and labour saving strategy.

The mealybug genus *Phenacoccus* is a widely distributed genus and has 206 species under it recorded throughout the world. However, in southern Asia, only 14 species have been recorded under this genus. Williams (2004) while describing species occurring in southern Asia, listed 40 species of mealybugs, that could become serious pests, if accidentally introduced elsewhere. *Ph. solani*, *Ph. madeirensis* and *Ph. solenopsis* were the species that were considered to be potential invasive species in his review. These species were found potentially invasive in India. These pests were reported from cotton, ornamental and weed plants (Anon, 2013a). In the present study similar mealybug species were recorded from the fruits, vegetables, ornamentals, medicinal, plantation crops and also on weeds. *Phenacoccus solenopsis* and *Ph. solani* are closely related species. In fact, it has also been recorded in India (Suresh and Kavitha, 2007), but Thomas and Ramamurthy (2008) considered it was a misidentification and stated it to be a phenotypic variation of *Ph. solenopsis*. They supported their view by studying large samples collected from different hosts and localities. In recent surveys, it was found that mealybugs *Ph. solenopsis* and *Ph. madeirensis* were attaining major status in horticultural ecosystems.

In India, the mealybug *Ph. parvus* was reported on China aster, *Callistephus chinensis* (L.) Nees (Asteraceae) (Sridhar et al., 2012). However, in the present investigation infestation of *Ph. parvus* are recorded even on vegetables along with ornamentals. The indication that this pest is spreading very rapidly to other hosts also brings a compulsory need to exercise management options before its occurrence reaches alarming proportions.

Other species of *Phenacoccus* viz., *Ph. divericatus* and *Ph. solani* are low risk quarantine pests that have been reported from Bengaluru in present investigation. But in future these species may attain a leading stage on most of the horticultural crops as mealybugs are polyphagous. Though there are some insecticides showing promising result, it is not expected very effective. This is due to the fact that mealybugs colonize in protected areas and insecticides do not reach to these areas effectively.
Natural enemies are very effective in reducing high mealybug populations, and minimising population build-up (Shylesha et al., 2010). Many natural enemies were recorded from the mealybug *Ph. solenopsis* and *Ph. madeirensis*, namely *A. amnestos*, *A. arizonensis*, *P. unfasciativentris*, *S. epius*, *S. croccivora* and *N. regularis* during the study period. These mealybugs could be controlled by these natural enemies effectively.

*Pseudococcus longispinus* is distributed worldwide and it has been reported as a pest in greenhouses, nurseries and fields in warmer areas. Severe infestation was reported on black pepper in India (Anon, 2010). During the study period, this species was recorded in lesser numbers on fruit crops as compared to ornamentals. Heavy infestation was found on jasmine in Malnad regions. Avocado plantations next to cotton fields were badly infested by *Ph. longispinus*, resulting in leaf-drop and a considerable percentage of fruits being damaged by the resulting honey dew. Effective control has been accomplished using biological control agents (Swirski et al., 1980). Natural enemies *Signiphora* sp. *Leptomastix* sp. *C. terebratus*, *L. shafeei* and *Mallada* sp. were recorded from the mealybug infested ornamental plants. A parasitoid *Anagyrus* sp. was recorded from the mealybug infested mango.

Mango is the main horticultural crop of India and the mealybugs occurring on it assume significance. Mealybugs usually starts infestation from the shoot but gradually migrate to the fruits (Chrysanthus, 2012). Fruits invariably drop or get discoloured due to stains from sooty mould. So controlling the pest before fruit formation is very important. In early stages pruning is the best method (Geiger, 2001) followed by release of the predator *C. montrouzieri* (Mani et al., 1995).

The distribution of *R. iceryoides*, *R. invadens* and *R. mangiferae* were studied on mango and were reported especially on abaxial and adaxial surfaces of leaves (Narasimham, 1990). The mealybug *R. iceryoides* known as one of the serious pest of mango in India causes huge damage (Tandon and Verghese, 1985). In the present investigation period, the mealybug *R. iceryoides* population in mango orchards was found to peak during summer from April to June. The infestation of *R. iceryoides* was seen on fruit stalk and fruits than leaves. This mealybug species was found more common than the other *Rastrococcus* sp. in almost all important mango growing provinces of Karnataka. Some of the natural enemies have been recorded on *R. iceryoides* in Karnataka and Uttar Pradesh, up to 42% parasitism was noticed (Tandon and Lal, 1978; Tandon and Srivastava, 1980). In the present studies parasitoids reported viz., *A. quadrii*, *A. chrysos*, *Coccophagus* sp., *Anagyrus* sp., *P. unfasciativentris*, *P. viridis* and *Leptomastix* sp. were found attacking on mealybug, *R. iceryoides*. The obtained
results are partially in agreement with the results of Narasimham and Chacko (1991). The predators *Leucopis* sp., *S. epius* and *C. perspicax* were reported on mealybug *R. iceryoides* infesting mango. The predator *C. perspicax* was found to feed more commonly on *R. iceryoides*. Hence the mass multiplication of this predator *C. perspicax* is essential to manage the mealybug. This mealybug is polyphagous in nature hence, in the present study it was also recorded on sweet orange, curry leaves, jamoon, karonda and pongamia.

In early 1980's *R. invadens* was accidentally introduced into several African countries, where it has became a serious pest of mango (Vogele *et al.*, 1991). In 1989 at the eastern border of Cote d' Ivory, the mango mealybug *R. invadens* rapidly became a nationwide restraint in production of mango. The infested orchards were destroyed by farmer due to 100% yield loss. This pest was reported for the first time in Bengaluru (India) on mango (Narasimham, 1990). This mealybug then started spreading slowly to even other mango belts. In the present study this mealybug species was collected in south and Malnad regions of Karnataka on mango. The population of *R. invadens* was moderately seen in Bengaluru and Malnad regions of Karnataka on mango. The preventive measures are very much necessary before the pest can reach its peak. To suppress the population of *R. invadens* the parasitoid *G. tebygi* and *A. mangicola* are used as biocontrol agents in Africa (Vogele *et al.*, 1991). In the present study the parasitoids *Anagyrus* sp., *Cheiloneurus* sp. and *P. unfasciativentris* were collected from *R. invadens* infesting mango in Karnataka. The mass production of these natural enemies is the most crucial factor for management of this mealybug species in future.

During the present investigation the mealybug *R. mangiferae* was recorded on shoots of mango in Bengaluru. It can be a low risk quarantine pest. This mealybug species was never found in other mango growing regions of Karnataka during investigation. This is a very important finding, as from here it can be a source of spread to neighbouring mango belts in Kolar, Chittoor, Dharmapuri and beyond. So fruit movement as well as nurseries should be kept under surveillance. Barcodes are also worked out in this study for quick identification of the mealybug.

5.5. Population dynamics of mango mealybug *Rastrococcus iceryoides* along with prediction models

Mealybugs belong to the family Pseudococcidae and have a world wide distribution, except in Arctica and Antarctica. They are dimorphic, the males being two-winged while females are wingless.
Mealybugs are one of the dreaded group of pests in horticulture occurring on almost all parts of the plant: buds, foliage, shoots, fruits, flowers, roots, rhizomes etc. They are usually wind borne as crawlers (the first instar) and drift and settle on plant parts of a host of vegetables, ornamentals and fruit crops. On settling they become sessile and spend rest of their feeding cycle, sucking sap. They also secrete a mealy powdery wax over them, which makes insecticidal interventions impervious. The colossal loss in horticultural industry suffered due to mealybugs as it is not adequately documented. Yet few examples, convey the importance of documenting and identifying mealybugs correctly using digital, molecular and morphological methods. One of the best examples of an invasive mealybug that ruined the horticulture industry was papaya mealybug *P. marginatus* which caused a loss of nearly Rs. 2000 crores every year (Anon, 2007). The first step in managing it was the correct identification of the mealybug (Miller and Miller, 2002). This helped in prompt importation of the parasitoid *Acerophagus papayae*, *Pseudleptomastix mexicana* and *Anagyrus loecki* which eventually controlled the mealybug pest on papaya in India (Shylesha *et al*., 2010).

One of the major pests of mango is *R. iceryoides*. So, specific monitoring of this was carried out. The data collected showed that the mealybug occurred most during the reproductive phase of the mango, starting from flowering to fruiting, till harvest. The maximum mean population of > 30 mealybugs / shoots or fruit was seen from end of April to end of May. The population studies is very important as it not only affects development of mango fruit, but the resulting sooty mould on the fruit reduces the commercial value and makes the fruit vulnerable to secondary infections (Pieterse *et al*., 2010).

Between March to June, when their numbers increased, at least 3- 4 generations can be expected considering that development takes 28-30 days according to Willink and Moore, (1988). They also say that each female produces about 160-190 crawlers, with females living up to three months. Therefore, a high and prolonged fecundity generates high population of mealybug by May, the peak season of mango fruits. Further, it was found that higher temperatures favour the mealybug. It was interesting to see that the mealybug population decreased after fruiting. Perhaps the nutrition in the fruit is what attracts the mealybug to mango. However, during vegetative phase small level of population occurred, which was probably the primary source of infestation subsequently during fruiting.

It is well known that mealybugs are associated with ants. As a part of documentation it was found that nine species of ants were associated with the mealybug for honeydew. The significance of ant association lies in the fact that it promotes *R. iceryoides* by maintaining
colony hygiene and being antagonistic to predators and parasitoids of mealybugs. In fact, ant numbers increased with mealybug population, signifying that ants opportunistically exploited the honeydew resource of the mealybug, as the latter population increased and correspondingly mealybug also benefitted.

The influence of abiotic factors on the mealybug population *R. iceryoides* was studied during 2012 - 2015. The population of *R. iceryoides* was found to be very low at the beginning of the mango season. Subsequently the population built-up, as the mango season advanced, reached its peak during mango harvesting which coincided with summer. These results agreed with the findings of Chrysanthus, 2012 who worked on seasonal variation of population dynamics of *R. iceryoides*. The cassava mealybug *Phenacoccus manihoti* Matile – Ferrero population was also found to increase during dry season compared to rainy season (Le Ru *et al*., 1991). According to Calatayud *et al*., (1994), drought-stressed fruits of cassava composed of nutrients such as sucrose and amino acids were either more concentrated or better balanced and such plants were more suitable for the development and reproduction of mealybug *Ph. manihoti*. This also probably explained the reason for high population of *R. iceryoides* on mango fruits during summer, as fruits are physiological sinks for the nutrients.

Population of mealybug *R. iceryoides* was positively correlated with maximum temperature and minimum temperature. Similar findings have been reported in other mealybug species *M. hirsutus* (Mani and Thontadarya, 1987, Prasanna and Balikai, 2015), *P. marginatus* (Seni and Sahoo, 2015) and *Ph. solenopsis* (Mandal *et al*., 2014). Similar trends of association between these weather parameters and population of the mealybug *R. iceryoides* was reported by Suresh and Kavitha (2007). According to them the high temperature and lack of rainfall were associated with an increase in mealybug population. They also found that for every unit increase in relative humidity and rainfall, there was 0.05 unit population reduction in the *R. iceryoides* population in mango. On the other hand every unit of sunshine hours increased the population by 3.93 units. Perhaps the mealybug showed significant positive correlation with maximum temperature, because temperature plays a vital role in faster multiplication of mealybugs, as quoted above.

In spite of this knowledge, there has been a missing link in Integrated Pest Management (IPM) decision - making, in the absence of a suitable prediction for *R. iceryoides* in mango. Once infested the fruit has to be discarded as no insecticidal treatment is useful, as they are inefficacious as well as residue - causing. However, biological control, using *C. montrouzieri* is recommended (Mani *et al*., 1995), which works only at early establishment with small populations. A prediction model, which predicts three to four weeks in advance of a potential
Infestation will help farmers gear up with biological control. This is imperative as obtaining sufficient numbers of *C. montrouzieri* requires three to four weeks. Thus, the present study of developing a prediction model is a useful addition to time biocontrol intervention for *R. iceryoides* control on mango fruits. Models which can predict *R. iceryoides* population with a single independent variable has the advantage of being used early by progressive farmer and extension personnel. Therefore, the outcome of this three-year study is bound to help in managing *R. iceryoides* at early incidence through biocontrol intervention.

### 5.6. Distribution of *Rastrococcus* species in major mango growing regions of Karnataka

In the present investigation, all the mealybug species were recorded during fruiting season of mango which coincides with summer in Karnataka. Therefore, temperature is probably the most important factor influencing abundance and distribution of insects (Saultani et al., 2012). The distribution of *Rastrococcus* species in major growing regions were studied during the study period.

In India, about 20 species of mealybugs are known to attack mango of which *R. iceryoides* is one of the major pests that causes significant damage (Tandon and Verghese, 1985). In present investigation *R. iceryoides* was recorded in all major mango-growing regions of Karnataka. Hence, this mealybug is considered as a primary mealybug pest attacking mango and widely distributed in India (Narasimham and Chacko, 1991). The infestations causing serious damage to mango in the Lucknow districts (Tandon and Lal, 1978). Major infestation was observed on fruits and fruit stalk than shoots. The peak population of *R. iceryoides* was recorded during fruiting season of mango. The nutrient composition of drought-stressed fruits such as sucrose and amino acids, either more concentrated or better balanced fruits were more suitable for the development and reproduction of mealybug species (Calatayud et al., 1994). This explains the reason why high population of *R. iceryoides* was found during mango season. *Rastrococcus iceryoides* is highly polyphagous in nature, as it was recorded on several host plants namely pongamia, red gram, karonda, curry leaf other than mango. This indicated that *R. iceryoides* occur throughout the year and ensures that this pest is capable of surviving on a wide range of hosts during off season. Hence, this species was found more dominant compared to other *Rastrococcus* species in mango ecosystem.

*Rastrococcus invadens* was found moderately during the study period and this mealybug does not seem to be of great economic importance in India (Anon, 2019). But this mealybug became a pest of major consequence on mango in Africa with yield losses estimated at 89%
in Benin (Bokonon-Ganta et al., 2002). If proper management care is not taken it may attain a major pest status on mango in India. In present studies *R. invadens* was recorded in southern and Malnad region of Karnataka on mango. According to Kemabonta and Odebiyi (2001), *R. invadens* nymphs had utmost survival and shortest developmental period on mango (21 and 24 days for ♀ and ♂ respectively) as compared to the growth of citrus – fed nymphs was the longest (26 and 24 days respectively). So mango is the most preferred host for *R. invadens*.

In the present investigation the mealybug *R. mangiferae* was recorded on mango only in Bengaluru urban. This finding agrees with the results of Narasimham (1990). Interestingly, it was observed that most of the population of mealybug *R. mangiferae* was found mummified due to parasitization. This may be one of the reasons that *R. mangiferae* is slow in spreading.

The overall studies showed that, an integration of molecular and morphological methods help in accurate identification of mealybugs, a prerequisite to ecological and management studies using the case of *Rastrococcus*, this study showed how biotechnological tools can help in pest predictions and control, and thus proving useful to farmers. Further, from quarantine and invasive mealybugs points of view, biotechnological identification will even precede morphological tools, as the former is faster, accurate and entails only a minimal specimen samples.