ECO-FRIENDLY MANAGEMENT OF PINEAPPLE MEALYBUG *Dysmicoccus brevipes* (COCKERELL) (HEMIPTERA: PSEUDOCOCCIDAE)

By
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(2014-11-214)

THESIS

Submitted in partial fulfilment of the requirement

for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR – 680 656

KERALA, INDIA

2016

DECLARATION

I hereby declare that the thesis entitled **"Eco-friendly management of pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae)"** is a bona-fide record of research work done by me during the course and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or society.

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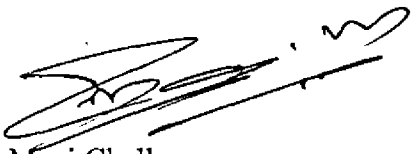

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ACKNOWLEDGMENT

Words are inadequate to express my sincere gratitude to my major advisor, ***Dr. Mani Chellappan****, professor and head, AINPAO, College of Horticulture, Kerala Agricultural University, Thrissur for his expert guidance, critical and valuable suggestions, constant encouragement and unfailing patience. His concern for my growth as a professional and as an individual has always transcended the boundaries of the workplace. He had tremendous faith in my ability to overcome what seem to be log in my journey.*

I consider myself gifted and privileged to have ***Dr. Madhu Subramaninan****, Associate Professor, AICRP on BCCP & W, Kerala Agricultural University, Thrissur as my Co-advisor, for being supportive and the friendly approach throughout this journey. His doors were always open for me and he always provided kind and insightful advice. His logical thinking, theoretical and practical knowledge of entomopathogenic fungi and constructive criticism helped me turn into a professional during the course of investigation (Thanks a lot sir).*

I profusely thank ***Vidya C. V.****, Assistant professor, AICRP on BCCP & W, Kerala Agricultural University, Thrissur (my best senior). She always enlightened me with her constant support and being patience listener of my worries and giving enough strength and courage to complete my research work. (Thanks a lot mam)*

I also extend my sincere gratitude to ***Dr. K. R. Lyla****, professor and former head of AICRP on BCCP & W, Kerala Agricultural University, Thrissur, Kerala Agricultural University, Thrissur for her whole hearted support and moral advisor who laid foundation for development of this research topic.*

It's my fortune to gratefully acknowledge ***Dr. Maicykutty P. Mathew****, Professor and Head, Department of Agricultural Entomology for her insightful, timely and valuable advice during the course of investigation and my PG programme.*

I consider it as my privilege to express my deep felt of gratitude to ***Dr. Babylatha,*** *Professor and Head, Pineapple Research Centre, Kerala Agricultural University, Thrissur and member of my advisor committee for her valuable advisor, motivation and encouragement during the course of my work.*

I express my deep sense of gratitude to all my dear teachers in the Department of Agricultural Entomology ***Dr. R. Ushakumari, Dr. Susannamma Kurien, Dr. Haseena Bhaskar, Ms. P. Sreeja, Dr. Smitha, M. S., Dr. Deepth,y K. P. and Dr. Berin Pathrose,*** *for the courage and enthusiasm they instilled in me.*

I am genuinely indebted to ***Dr. Krishnan,*** *Professor and Head, Department of Agricultural Statistics for his valuable assistance and guidance during the statistical analysis of the data.*

I avail this opportunity to express my deep sense of gratitude and sincere thanks to ***Dr. P. P. Joy,*** *Professor and Head, Pineapple research station, Vazhakulam and* ***Mr. Joy Mathew*** *(farmer) Vazhakulam for his whole hearted help extended throughout the period of my survey.*

Words are inadequate to express my deep sense of gratitude to my besties ***Neenu Chandran, Uma Maheswari, Chandini S. M. Indraji, Rekha, Ashwini, Shobha, Kusuma, Yan singh, Supritha*** *and others for the support and courage they gave during the difficulties of my work and being supportive during all my ups and downs. They were like oasis in desert and made the journey most memorable.*

I would like to thank my seniors my seniors ***Harish E.R,*** *Neena Lenin, Najitha Ummer, Nasmi, Maheswari, Nasiya, Neethu and Aswin who always there during my needs and also for their advice and support for the successful completion of PG programme. I thank* ***Nithya chechi,*** *who became closer to me within less span of time, her friendly nature, honest advice and valuable suggestions helped me a lot during the investigation.*

*I wish to express my sincere gratitude to **Sujala chechi** (AINPAO) for her unfailed patience and helping me in taking beautiful photos of the specimen. She had been a lovable sister and showed unconditional love towards me. I thank Juvin, Bajith, Sruthi and others (AINPAO) for helping me during the survey. I would also like to thank **Akila chechi** (BCCP) for helping to carry out my laboratory work and to all the labourers who helped in successful completion of my field work.*

*Words cannot express my indebtedness to my family - **pappa, amma** and **Deepu** for being supportive and strength given to me. It was hard to complete these two years without you people. Pappa, you are my strength and amma is inspiration, I am nothing without you and what I am today is all because of your scarify and having faith in me. Lost but not least I thank all my UG teachers and my besties **Ramya (pakoda), Swetha, Muniswami, Murali, Vijay, Venki anna** and my sweet juniors **Srikanth, Lashmimitra, Nithya** and **Prabhakar** for their unconditional love and being supportive during my falls and keeping the spirit in me.*

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Introduction

1. INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) is a tropical fruit plant belonging to the family Bromeliaceae. It is originated from regions of Southern Brazil and Paraguay. Fruits are either consumed fresh or used for making juice. It is an excellent source of potassium (109 mg/100g), calcium (12mg/100g), magnesium (12mg/100g), vitamin C (47.8mg/100g), carbohydrate (13.12 g/100g) and sugar (9.85 g/100g). Pineapple fruit is the only source for naturally available bromelain which is used for healing cancer, wounds and inflammation as well as in enhancing the immune system. In addition to this, pineapple leaves are used in textile industries for fibre production.

Pineapple is one of the most important fruit crop grown in India, covering an area of 1,09,900 ha with an annual production of 1736.70 metric tons. Assam has the largest area under pineapple (16.54 thousand ha), followed by Manipur (13.70 thousand ha) and Arunachal Pradesh (12.78 thousand ha). West Bengal ranks first in production (316 metric tons) and is followed by Assam (288.60 metric tons) and Tripura (162.26 metric tons). Kew, Giant Kew, Queen and Mauritius are the popular varieties cultivated in India.

Kerala with an area of 8.54 thousand ha and 72.86 metric tons production contributes about 4.2 per cent of the pineapple production in India. The major pineapple growing areas in Kerala are Moovatupuzha, Kothamangalam in Ernakulam, Thoduphuza and Elamdesam in Idukki district and parts of Kottayam district (NHB, 2014).

Many insects are known to attack pineapple, but only a few are considered as major pests, such as pineapple mealybug, *Dysmicoccus brevipes* (Cockerell), scale insects [*Diaspis bromeliae* (Kerner), *Parasaissetia nigra* (Nietner) and *Melanaspis bromeliae* Dekle] and thrips [*Thrips tabaci* (Linderman), *Frankliniella schultzei* (Trybom) and *Halothrips ananasi* Costa Lima]. Among these, infestation by pink pineapple mealybug, *Dysmicoccus brevipes* often leads

to complete devastation of the crop, as the insect also acts as vector of Pineapple Mealybug Wilt Diseases (PMWD). It was reported as a serious pest of pineapple in Kerala (KAU, 2002)

Two species of pineapple mealybugs are known to occur *ie.,* the pink strain (*D. brevipes*) and the grey strain (*D. neobrevipes*). Pink strain is mostly found on the root, crown and lower stem of pineapple plants and reproduces parthenogenetically. The grey strain, on the other hand, mostly seen on upper parts of the plant, such as leaf whorls and the developing fruits and reproduces sexually (Ito, 1938).

Pink mealybug, *D. brevipes* consists of two races, parthenogenetic race and bisexual race. Parthenogenetic race differ from the bisexual race being commonly found on upper parts of the plants and by producing green coloured spots on the infested pineapple leaves. Bisexual race has been first observed on pineapple in Brazil, Dominican Republic, Martinique, Malaysia, Madagascar and Ivory Coast (Beardsley, 1993). Both pink strain and grey strain mealybugs are responsible to transmit PMWD in Hawaii. But the grey strains are restricted only to tropical America and Hawaii. Apart from pineapple, crops like coffee, banana, caladium, canna, citrus, eggplant, sugarcane and palms are reported as alternate hosts of pineapple mealybugs (Hara *et al.*, 2001).

Since, *D. brevipes* is a highly polyphagous pest and a major constrain to pineapple cultivation, management of this mealybug is very important. Use of synthetic insecticides for the management of mealybugs results in residual toxicity in fruits and may cause human health hazards, besides eliminating the natural enemies which play a crucial role in bringing down the population of mealybugs. Therefore, an effective and ecologically sound management practice of pineapple mealybugs has to be developed. Hence, the present study is proposed with the following objectives:

1. To document the natural enemy fauna of *Dysmicoccus brevipes.*
2. To formulate eco-friendly measures for managing the pest population.

Review of literature

2. REVIEW OF LITERATURE

Pink pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Pseudococcidae: Hemiptera) has been regarded as a cosmopolitan pest of *Ananus comosus* (L.). This is a polyphagous pest found infesting various fruit crops, pulses, perennial weed plants, *etc.*, and are associated with the pineapple mealybug wilt disease transmission. The available literature related to host range, biology, economic importance and management of *D. brevipes* and other mealybugs are reviewed in this chapter.

2.1 Distribution and host range of pink pineapple mealybug, *Dysmicoccus brevipes*

Poinar (1964) reported the presence of *Dysmicoccus brevipes* on roots of purple nut grass, *Cyperus rotundus* L. and a closely related species, *C. esculentus* L. The first report of *D. brevipes* infestation on the root nodules of red gram (*Cajanus cajan* L.) and ground nut (*Arachis hypogea* L.) was from Southern India. About 80 per cent of the plants were found affected (Rajagopal *et al.*, 1982). Mani and Thontadarya (1987) identified four species of mealybugs infesting grapevine *viz.*, *Planococcus citri* (Risso), *Maconellicoccus hirsutus* (Green), *Nipaecoccus viridis* (Newstead) and *Dysmicoccus brevipes*. Among these, *D. brevipes* was considered to be minor importance and supposed to be the first report of this pest on grape.

Dhileepan (1991) conducted a survey in oil palm growing regions of Andhra Pradesh, Karnataka, Kerala and Maharashtra and observed the incidence of *D. brevipes* on fruit bunches of oil palm at Palode, Kerala and classified it as a potential pest on oil palm. Hara *et al.* (2001) reported that *D. brevipes* had gained the status of quarantine significance in Hawaii as it was found on the exposed roots of pineapple and various crops like coffee, banana, caladium, canna, citrus, eggplant, sugarcane and palms. Radhakrishnan *et al.* (2003) reported *D. brevipes* for the first time in Kerala, infesting the immature nuts of coconut.

Culik and Gullan (2005) recorded the occurrence of *D. brevipes* on sugarcane and pineapple from Espirito Santo, Brazil. They also collected few mealybugs from pumpkin (*Cucurbita pepo* L.) in Vitoria. Torres and Avila (2005) found sporadic incidence of *D. brevipes* in Nayarit, Mexico on organically grown coffee, garden vegetables, medicinal herbs, *etc.*, though no significant damage was recorded on infested crops. According to Maghaddam (2006) *D. brevipes* was found to infest sugarcane and rice periodically and reported it as potential pest of crops which were being cultivated in southern part of Iran. *D. brevipes* was reported to be introduced to Iran from Philippines and other countries of East Asia through crops like banana, pineapple and ornamental plants.

Martinez *et al.* (2007) noticed the incidence of *D. brevipes* on coffee and cacao plants. In Kerala, it was reported to infest the roots and basal stem region of pepper (Devasahayam *et al.,* 2009). Hernandez and Martinez (2012) reported the incidence of *D. brevipes* for the first time on rhizome of white ginger flower, *Hedychium coronarium* Koenig in Cuba. Infested plant showed the characteristics symptom of necrotic depressions, where the different stages of mealybugs were found. Basavaraju *et al.* (2013) studied the seasonal abundance of *D. brevipes* on arecanut (*Areca catechu* L.) in Karnataka and they observed higher population during December-July and severe during March-May. Usually the incidence of mealybug was abundant on nuts while it was occasionally found on leaves and inflorescence.

Vijay and Suresh (2013) gave an account of the incidence of *D. brevipes* for the first time on *Cassia occidentalis* L. and *Ocimum sanctum* L. in Tamil Nadu by conducting survey in different districts, where the flowers and medicinal plants were grown. Among the surveyed areas, mealybug population was found to be very low on *C. occidentalis* and *O. sanctum* in Pechiparai and Ooty while in the other areas it was more pronounced. Filho *et al.* (2015) reported the incidence of *D. brevipes* on grapes in Brazil. About seven species of mealybugs were noticed on grape bunches, among which the incidence of *D. brevipes* was recorded as low (3 %).

2.2 Economic importance of *Dysmicoccus brevipes*

D. brevipes acted as vector in transmitting Cacao Mottle Leaf Virus (CMLV), Cacao Swollen Shoot Virus (CSDV), Cacao Trinidad Virus (CTV) (Harris, 1981), mealybug wilt disease in Srilanka (Hughes and Samita, 1998) and Banana Streak Virus (BSV) in Uganda (Kubiriba *et al.*, 2001). Sether *et al.* (2001) conducted a survey of pineapple plants from Hawaii and other places all over the world, revealed that *D. brevipes* carried two types of viruses namely, Pineapple Mealybug Wilt associated Virus-1 (PMWaV-1) and Pineapple Mealybug Wilt associated Virus-2 (PMWaV-2) while Sether and Hu, 2001 observed the pineapple infected with PMWaV-1 produced fruits with reduced size in USA (Sether and Hu, 2001).

JyeYann *et al.* (2008) collected samples of pineapple plants from different regions of Central Taiwan. Affected plants showed the typical symptoms such as tip dieback, reddening and downward curling of leaf margins, early stage of green bumpson leaves, fruits with mealybugs and covered with sooty mould. Electron microscopic observations of infected samples revealed that plants were infected with PMWaV-1. Hernandez *et al.* (2010) confirmed transmission of PMWaV-3 disease by *D. brevipes* in Cuba. It caused a serious economic problem in pineapple growing areas with 40 per cent reduction in yield. Incidence of PMWD was higher during the dry season in Central Uganda. Severe incidence of disease was found associated with mealybug population. Infected plants showed yellowing of leaves, stunting, wilting and rotting of roots followed by reduced yield and low plant population (Bua *et al.* 2013). Alvarez *et al.* (2015) for the first time reported that *D. brevipes* transmitting PMWaV-1 in Ecuador.

2.3 Biology of *Dysmicoccus brevipes*

Ito (1938) studied biology of *D. brevipes* and documented the average duration of first, second and third instar nymphs as 14, 9.8 and 10.3 days, respectively, while the adult female longevity was about 55 days with pre-larviposition period of 27 days, larviposition period of 25 days and post-

larviposition peroid of five days. No males were observed among the population and females reproduced parthenogenetically. Bionomics of the bisexual races of *D. brevipes* was studied by Lim (1973) in Malaysia. Under laboratory conditions, it was observed that the life cycle of bisexual races were relatively shorter than the parthenogenetic races of *D. brevipes* in Hawaii. The females had three nymphal instars with each instar lasting for 10, 6.7 and 7.9 days while males had two nymphal instars followed by prepupal and pupal stages lasting 9.9, 5.8, 2.5 and 3.7 days, respectively. The adult males lived for one to three days and adult female for 17-49 days with pre-larviposition, larviposition and post-larviposition period of 14.6, 9.1 and 4.3 days, respectively.

Ghose (1976) studied the biology of parthenogenetic races of *D. brevipes* on sprouted potato tubers under laboratory conditions at temperature of 30° C and 66 per cent relative humidity, observed that the nymphal stage lasted for an average of 19.27±1.94 days, the pre-oviposition period for 16.37±1.36 days, and ovipositition period for 40 days. The mean fecundity was 240 eggs per female. Basavaraju *et al.* (2013) examined the biology of *D. brevipes* in laboratory on coconut leaf bits in Karnataka indicated the presence of three nymphal instars in both males and females with the presence of a pupal stage in males with a total life-cycle of male and female as 24.88±2.21 and 25.38±0.95 days, respectively.

Effect of host plants on the development, survival and reproduction rate of *D. brevipes* on grapevine varieties carried out by Bertin *et al.* (2013) revealed the presence of three nymphal instars in females, which reproduced by thelytokous parthenogenesis. No males were observed. There was no difference in development period of first and second instar nymphs on leaves and rootstock of the varieties Italia and Niagara rosada. However, a significant difference was observed in duration of third instar nymphs when reared on different rootstocks with longest duration being on Niagara Rosada (47.23±39) when compared with Italia (42.20±1.07).

2.4 Ant-mealybug interaction in pineapple fields

Phillips (1934) observed an association between mealybugs and ants in pineapple fields. Mealybugs were protected from natural enemies in the presence of ants and they also were involved in the dispersal of mealybugs from plant to plant. Ants also prevented the growth of fungi by removing the honeydew. Rajagopal *et al.* (1982) reported that the ant, *Monomoruim* sp. was observed to be attracted towards the *D. brevipes* infesting on the root nodules of red gram and groundnut. The big headed ants, *Pheidole megacephala* (F); fire ants, *Solenopsis geminata* (F) and the Argentine ants, *Linepithemia humuli* (Mayer) were considered as the most important ant species due to their close association with *D. brevipes* (Reimer *et al.*, 1990).

The population of mealybugs was found to be the highest in pineapple fields, where the big headed ants, *P. megacephala* were present. Complete elimination of ants from the field resulted in reduction of mealybugs population. In the presence of ants, pineapple plants were prone to PMWD (Petty and Tustin, 1993). A survey was conducted by Gonzalez-Hernandez *et al.* (1999) in pineapple growing region of Hawaiian Islands. Five natural enemies *viz.*, *Anagyrus ananatis* Gahan, *Euryrhopalus propinquus* Kerrich, *Lobodiplosis pseudococci* (Felt), *Nephus bilucernarius* Mulsant and *Sticholotis ruficeps* Weise were associated with *D. brevipes.* In the presence of ants, density of natural enemies was low (<10 individuals/plant) and their efficiency was also found to be reduced.

According to Jahn and Beardsley (2000), *D. brevipes* was often subterranean and the fire ants, *Solenopsis geminata* were reported to build nests around colonies of mealybugs in Hawaii. Control of ants in the pineapple fields resulted in significant reduction of mealybugs and incidence of PMWD (Yan-biao *et al.*, 2013).

2.5 Eco-friendly management strategies of mealybugs

2.5.1 Cultural and mechanical control

According to Ishaq *et al.* (2004), in Pakistan non-chemical management practices were found to be superior in reducing the mealybug (*Drosicha stebbingi* Green) and fruit fly (*Dacus zonatus* Saunders and *Dacus dorsalis* Hendel) on mango. Mortality rate achieved with insecticides were up to 55 per cent. Burlap bands and methyl eugenol traps gave a best control of mealybugs and fruit flies with 78.98 and 98 per cent mortality, respectively. According to Shrewsbury *et al.* (2004) spraying of insecticides failed to reduce the population of citrus mealybug, *Planococcus citri*. Regulation of water and fertilizer along with augmentation of *Cryptolaemus montrouzieri* Mulsant remarkably reduced the population of mealybugs (ranging from 78 to 98 per cent).

Manjunath *et al.* (2006) studied the role of trap crops in the establishment of pest and the predators in mulberry field. Out of four trap crops like *Gliricidia sepium* (Jacq.), *Abelmoschus esculentus* (L), *Hibiscus cannabius* (L) and *Croton* sp. used, *H. cannabius* was found effective. There was a significant decrease in 'Tukra' incidence in mulberry with the trap crop (4.28±2 per cent reduction) than mulberry field without trap crop (11.44±2.93).

According to Mandal *et al.* (2009) integration of various management practices like weeding, use of botanicals and chemicals reduced the PMWD incidence and mealybugs to a great extent (2.62 per cent) with the higher yield (41.6 t/ha). Insecticides alone reduced the mealybugs to 11.88 per cent with the minimum yield of 32.5 t/ha. Insecticides combined with botanicals (neem oil) recorded the reduction in mealybugs up to 38.71 per cent with the yield of 32.5 t/ha. Karar *et al.* (2010) reported mango mealybug, *Drosicha mangiferae* Green was effectively controlled by mechanical and cultural methods. Mechanical bands around the tree trunks above the ground level and hoeing or ploughing the orchard, earthing up or mounding the tree trunks with fine mud, dried leaves, debris and mud clods significantly reduced the mealybug populations.

Stripping the bark of the grape vines followed by swabbing with insecticide and banding the trunk with Stickum® was found effective in the management of mealybugs in grapevine yards (Daane *et al.*, 2012). According to Tachie-Monson *et al.* (2014) management of weeds in pineapple fields resulted in best management of mealybugs. Removal of weeds resulted in exposure of mealybugs to their natural enemies and also dislodged the ants which aided in spread of the mealybugs.

Kabi *et al.* (2016) studied the influence of farm type, cropping systems and soil management practices in controlling the population of pineapple mealybugs, *D. brevipes*. Mealybugs population densities were lower in pineapple-banana intercrop system (27.8 mealybugs/plant) than in a sole pineapple crop (81.8 mealybugs/plant). Earthed up seed beds registered higher mealybugs densities (84.1 mealybugs/plant) and created more favourable environment for mealybugs multiplication than flat seed beds (31 mealybugs/plant). Use of coffee husks as soil amendment promoted mealybugs population build up, whereas fallowing led to reduction in population.

2.5.2. Use of botanicals

Gupta *et al.* (2007) conducted a study under laboratory condition to evaluate the efficacy of two concentrations (1.0 and 1.5%) of methanolic leaf extracts of five different plants, oleander (*Nerium indicum* L.), ram tulsi (*Ocimum gratissimum* L.), marigold (*Tagetes erecta* L.), japanese mint (*Mentha arvensis* L.) and custard apple (*Annona squamosa* L.), against the mealybug, *Ferrisia virgata* (Cockerell). Study revealed that japanese mint proved to be more effective resulting in 100 per cent mortality after 96 hours at 1.5 per cent concentration and after 120 hours of treatment at 1.0 per cent concentration and it was significantly better than all other treatments. It was followed by custard apple (mortality 40.00-93.33% @ 1% and 46.66-100% @ 1.5% concentration), oleander (33.33-86.66% @1% and 53.33-100% @ 1.5% concentration) and ram tulsi (26.66-73.33% @ 1% and 40.00-93.33% @ 1.5% concentration). Marigold showed poor initial

results, but its mortality increased to 86.66 per cent at 1 per cent concentration and 93.33 per cent at 1.5 per cent concentrations, 120 h after treatment.

Patil *et al.* (2010) reported that the plant extracts of different parts of tropical plant, *Balanites aegyptiaca* (L) Del. was found effective against *Maconellicoccus hirsutus* (Green). Various solvent extracts of roots, methanol extracts of leaves, fruits, flowers and roots, partially purified saponins along with the commercial bark saponin extract from *Quillaja saponaria* Molina were used. The study revealed that methanol root extracts was the most effective at the concentration of 500 µg per litre when compared with other extracts. Longevity and oviposition of *M. hirsutus* was found to reduce greatly upon spraying of partially purified saponin of *B. aegyptiaca* and the commercial bark saponin extract from *Quillaja saponaria* and other extracts of plant.

Biopesticides like tobacco medicine (100%), tobacco leaf extract (20%), mahagoni seed oil (5%), castor seed oil (5%) and neem seed oil (5%) were found to be effective in controlling of papaya mealybug, *Paracoccus marginatus* (Williams & Willink) infesting vegetable crops in Bangladesh. Mortality rate varied with different biopesticides and the highest mortality was observed with tobacco medicine (93.43%) followed by neem seed oil (90.11%), mahagoni seed oil (80%), castor seed oil (78.92%) and tobacco leaf extract (68.37%) (Ahmed *et al.*, 2011).

Neem oil was found to be effective in management of *Phenacoccus solenopsis* Tinsley on cotton. Mortality per cent was less with neem oil over the synthetic insecticides. However, there was a significant reduction in mealybugs population with 54.28 and 69.63 per cent mortality (@ 1.5 and 2 %). Mortality rate was found to increase gradually with the high exposure period (Mamoom-ur-Rashid *et al.*, 2011).

Leaf extracts of indigenous native botanicals like *Azadirachta indica* A. Juss, *Eucalyptus globulus* L., *Ocimum basilicum* L. showed repellent property against *Phenacoccus solenopsis*. Mealybugs were sprayed with different

concentrations of botanicals like 1, 2, 3, 4 to 10 per cent. Highest repellence of 97 per cent was observed with leaf extracts of *A. indica* followed by 93 per cent with *E. globules* and 88 per cent with *O. basilicum* (Singh *et al.*, 2012). Under glasshouse condition, botanicals like Lastraw^{TM} (5ml/l) and neem oil 300 ppm were found to be effective in reducing the population of *P. marginatus* on mestha (*Hibiscus cannabinus* L.). Seven days after the spraying, the mortality of 78.74 and 76.19 per cent was found with Lastraw^{TM} and neem oil, respectively (Gowda *et al.*, 2013).

Halder *et al.* (2013) observed the median lethal time (LT_{50}) of different entomopathogenic fungus and neem oil and their combination with 1:1 ratio. *V. lecanii* was found most promising against 7±1 days old nymphs of *P. solenopsis* followed by *Beauveria bassiana* (Balsamo) and their corresponding LT_{50} value were 112.28 and 124.04 h, respectively. However, when neem oil 5 per cent was tested alone, LT_{50} was 93.71 h and it was the lowest. All the microbial insecticides were blended with neem oil (1:1 ratio) and found compatible. LT_{50} value was 87.67, 88.34 and 90.82 h for *Verticillium lecanii* Zimmerman, *B. bassiana* and *Metarhizium anisopliae* (Metch.), respectively.

Balasaraswathi *et al.* (2014) reported the effects of various organic cakes in inducing systemic resistance against *M. hirsutus* which caused 'Tukra' disease in mulberry. Different organic cakes were applied to mulberry plots separately along with FYM. The incidence of 'Tukra' disease was found least with FYM + neem cake (3.01%) succeeded by the FYM + pongamia cake (3.82%), FYM + mahua cake (3.94%), FYM + custard apple seed cake (4.12%) and FYM+ jatropha cake (4.43%), as against sole NPK application (16.21%). According to Prishanthini and Vinobaba (2014) plant extracts were found to be the most effective in reducing the population *P. solenopsis* on shoe flower plants, *Hibiscus rosa-sinensis* L. Out of various botanicals *O. sanctum*, *A. indica*, *Calatropis gigantia* L., *Nicotina tobaccum* L. and *Alium sativum* L. tested, *O. sanctum* gave desirable control of mealybugs.

Thinnaluri *et al.* (2014) used the seed kernel and leaf extract of *A. indica*, *Pongamia pinnata* L, *Madhuca longifolia* Macb and leaf extracts of *Lantana camera* L. and *Adathoda vasica* L. to manage the Tukra in mulberry. The highest reduction of Tukra incidence was recorded with the application of 4 per cent NSKE (92.10 per cent) followed by 2 per cent pongamia seed kernel extract (86.64 per cent) and 8 per cent lantana leaf extract (29.91 per cent).

According to Manzoor and Haseeb (2015) plant extracts like *Annona squamosa* (5%), neem excel (0.3%), neem leaf (5%), neem cake (5%), *Allium sativum* (5%), *Calotropis gigantea* (5%), *Ocimum sanctum* (5%) shown insecticidal property which was found to be effective against *P. solenopsis*. *Annona squamosa* showed a significant reduction in the mealybug populations with 31.4 per cent mortality after 24 h of application.

2.5.3 Biological control of mealybugs

2.5.3.1 Natural enemies reported on *Dysmicoccus brevipes*

Compene (1936) described an encyrtid parasitoid, *Hambletonia pseudococcinia* Compene on *D. brevipes* in Brazil. Pandey and Johnson (2006) conducted a survey in Hawaii to document the weeds on which *D. brevipes* colonized. It was noticed that the parasitoid, *Anagyrus ananatis* Gahan was abundant on *D. brevipes* collected from pineapple plants. But there was no report of mealybug from weeds.

Culik and Ventura (2009) described a new species, *Rhinoleucophenga capixabensis* sp. nov. (Diptera: Drosophilidae) as a potential predator of pineapple mealybug. Culik *et al.* (2011) recorded three species of chalcidoid parasitoids, *Adelencyrtus moderatus* Howard, *Homolapoda* sp. and *Diglyphomorpha* sp. infesting on scale insects in Brazil, among which *Diglyphomorpha* sp. was found to be associated with *D. brevipes*. The activities of parasitoid, *Anagyrus* sp. and predators, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) and *Scymnus* sp. were found to be high during April-May in Karnataka. Multiplication

of these natural enemies was favoured by high temperature and availability of *D. brevipes*.

2.5.3.2 Natural enemies on other mealybugs

Mani *et al.* (1995) conducted a study to identify the natural enemies associated with mango mealybug, *Rastrococcus iceryoides* Green and predatory efficiency of *C. montrouzieri*. About seven species of natural enemies were identified among which the parasitoids, *Anagyrus* sp. nr. *dactylopii* and *Coccophagus sexvittatus* Hayat and the predator, *Cacoxenus perspicax* (Knab) were observed newly. *C. montrouzieri* was recorded to feed an average of 355 eggs and 498 nymphs of mealybugs during its larval period. Field release of *C. montrouzieri* at 50 per plant resulted in significant reduction of mealybugs infesting the mango fruits.

Carver *et al.* (1987) conducted a survey to report the diversity of natural enemy fauna associated with *Saccharicoccus sacchari* (Cockerell) in sugarcane growing regions (Queensland and northern New South wales) of Australia. Large population of *Anagyrus saccharicola* Timberlaka (Hymenoptera: Encyrtidae) were collected from mummified mealybugs from the field. Other precocious population included larvae of *Cacoxenus perspicax* (Knab) (Diptera: Drosophilidae), larvae of *Coccodiplosis* sp. (Diptera: Cecidomyiidae), *Cryptolaemus montrouzieri* and *Oplobates woodwurdi* Gross (Hemiptera: Anthocoridae). Along with these an ectoparasitic and saprophytic fungi *Aspergillus parasiticus* Spear was found to reduce the population of mealybug. According to Neuenschwander (2001) an encyrtid parasitoid, *Apoanagyrus* (*Epidinocarsis*) *lopezi* De Santis was found to be effective in bringing down the population of cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero.

Exploration for natural enemies of pink hibiscus mealybug, *M. hirsutus* (Green) was done, which was a polyphagous pest in Australia. Mealybugs were collected at monthly intervals over a period of two years from different regions on various plants. The study found to recover large population *Cryptolaemus*

montrouzieri, predaceous drosophilid fly, *C. perspicax* which was found only at higher densities of mealybug, encrystid parasitoid *Gyranusoidea indica* Shafee, noctuid larvae, *Mataeomera* sp. and a hyperparasitoid, *Coccidoctonus* sp (Goolsby *et al.*, 2002) .

A survey was conducted by Kaydan *et al.* (2006) in Turkey to explore the natural enemies of different mealybug species. In the survey about 23 predatory and 22 parasitoid species of different insect orders were collected. Most of the predators belonged to the order Coleoptera (17 species) and few were from the order Diptera (3 species) and Neuroptera (3 species), while a large number of parasitoids recorded were from the order Hymenoptera belonging to the different families like Aphelinidae, Encyrtidae, Platygasteridae, Pteromalidae, and Signiphoridae. Among these, ten newly reported species of natural enemies were present which included *Sidis biguttatus* Motchulsky, *Nephus sinuatomac- ulatus* Sahlberg (Coccinellidae), *Leucopomyia alticeps* Czerny, *Parochthiphila* (*Euestelia*) *decipia* Tanasijtshuk (Chamaemyiidae), *Leptomastidea matritensis* Mercet, *Prochiloneurus bolivari* Mercet, *Rhopus* sp. nr. *acaetes* (Walker), *Stematosteres* sp., *Eunotus acutus* Kurdjumov, and *Chartocerus kurdjumovi* (Nikolskaya) (Chalcidoidea).

According to Mani and Krishnamoorthy (2007) spraying of dimethoate (0.05%) and monocrotophos (0.05%) against *Ferrisia virgata* (Cockerell) infesting tuberose, *Polyanthes tuberose* (Linn.) failed to control the mealybugs. However, release of *C. montrouzieri* (@ 850 larvae/170 sq meter) completely eliminated the mealybugs. *C. montrouzieri* was reported as potential predator for the elimination of mealybug species like spherical mealybug, *Nipaecoccus viridis*, *F. virgata* and *P. citri* infesting on pummelo to an extent of 97.74, 90.17 and 82.37 per cent, respectively (Mani and Krishnamoorthy, 2008).

According to Afifi *et al.* (2010) *C. montrouzieri* was found promising in the management of *P. citri* infesting croton ornamental shrub, *Codiaeum variegatum* L. One month after release of the predator, reduction in egg mass,

nymphs and adults of *P. citri* was found to be 80.6, 86.5 and 91.5 per cent, respectively, while other natural enemies associated were three predators (*Sympherobius amicus* Navas, *Scymnus syriacus* (Mars) and *Chrysoperla carnea* Stephens) and a parasitoid, *Coccidoxenoids peregrinus* (Timberlake). Thangamalar *et al.* (2010) reported *Spalgis epius* (Westwood) was effective in management of *Paracoccus marginatus*. Single larvae devoured about 42 to 53 ovisacs and 196 to 222 nymphs and adults of *P. marginatus*.

Menochillus sexmaculatus Fab., *Coccinella septempunctata* L., *Brumus suturalis* (Fab.) and *Hippodemia convergens* Guerin - Meneville were reported as potential predators on the first instar nymphs of *P. solenopsis*. All the life stages of these coccinellid bettles were found to be predaceous on mealybugs (Arif *et al.*, 2011). According to Dinesh *et al.* (2011), *S. epius* was a voracious predator on *M. hirsutus*. It was reported that a single larva consumed about 2358.3 eggs, 1512 nymphs and 34.3 adults of *M. hirsutus* during its development period. Sahito *et al.* (2011) recorded certain natural enemies *viz.*, *Aenasius bambawalei* Hayat (Hymenoptera: Encrytidae), *Brumus saturalis*, *Menochilus sexmaculatus*, *Scymnus coccivora* Ayyar, *S. suturalis* Thunberg and *Chrysoperla carnea* Stephens on *P. solenopsis* infested cotton.

In India, parasitisation of encyrtid parasitoid *Aenasius bambawalei* Hayat on *P. solenopsis* infesting cotton plants varied from 20 to 70 per cent. The efficiency of *A. bambawalei* was found to be reduced by the association of a hyperparasitoid, *Promuscidea unfasciatixentris* Girault (Hymenoptera: Aphelinidae) (Tanwar *et al.*, 2011). Aravind *et al.* (2012) reported, green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Petersen) feeding on two tailed mealybug, *Ferrisia virgata* infesting okra plants.

Kaur and Virk (2012) evaluated the feeding efficiency of *C. montrouzieri* against *P. solenopsis* found that fourth instar grubs and adults of *C. montrouzieri* were the voracious feeders on the first instars nymphs of mealybug. Rahmouni and Chermili (2013) studied the efficiency of the *C. montrouzieri* against *P. citri*.

C. montrouzieri was released in mealybug infested citrus orchard on two citrus varieties Maltaise and Maltaise Douse in the region of Mornag and El Gobba, respectively. Mealybug populations were reduced by 85 per cent in El Gobba and 89 per cent in Mornag orchard in one month after the release of predators. Third instar grubs of *Chrysoperla carnea* was reported to show the highest feeding efficiency on *P. solenopsis* (Hameed *et al.*, 2013). According to Parihar *et al.* (2015) prey consumption was higher in *Mallada desjardinsi* (Navas) than *C. carnea.*

Prasanna and Balikai (2015) assessed the seasonal incidence of grapevine bug, *Maconellicoccus hirsutus* (Green) and its natural enemies in Dharwad, Karnataka for two seasons recording the infestation throughout the year and reported the presence of seven predators, four coccinellids (*Scymnus nubilus* Mulant, *Nephus regularis* (Sicard), *S. coccivora* and *C. montrouzieri*) and two Dipteran families, Chamaemyiidae (*Leucopis* sp.) and Drosophilidae (*C. perspicax*) and one from lepidopteran family, Lycaenidae (*Spalgis epius*). Twelve parasitoids were recorded belonging to the order Hymenoptera *viz., Coccophages pseudococci* Compere, *Coccophagus* sp., *Promuscidea unfasciativentris* Girault and *Euryischomyia* sp. (Aphelinidae), *Aenasius bambawalei* Hayat, *Leptomastix dactylopii* Howard, *L. lyciae* Howard and *Homalotylus eytelweinii* Ratzeburg (Encyrtidae) and *Oomyzus* sp. (Eulophidae), *Centiste* sp. (Braconidae) and *Metastenus concinnus* Walker (Pteromalidae).

According to Persad and Khan (2002) the generation time of the natural enemies like *Anagyrus kamali* (2.09 days), *C. montrouzieri* (5.13 days) and *S. coccivora* (4.45 days) was found to be doubled the time required by its host *M. hirsutus* (8.83 days).

2.5.3.3 Microbial control of mealybugs

According to Kulkarni *et al.* (2003) different concentrations (2, 3, 4, 5 and g/l) of *Cephalosporium lecanii* Zimmerman were evaluated against *F. virgata* and *P. citri* on pomegranate in Rahuri. All the concentrations were effective for

controlling these mealybugs. However, on the basis of effectiveness, economics and persistency, dose of 4 g/l was found to be the optimum for the management of mealybugs on pomegranate. According to Chang-cong (2008) *Metarhizium* J813 strain was found effective against *D. brevipes* and showed the highest pathogenicity.

According to Katke and Balikai (2008) spraying of dimethoate 30 EC @ 1.7 ml + fish oil rosin soap @ 5 g/l, dimethoate 30 EC @ 1.7 ml/l, *V. lecanii* (WP) (@ 2.0 g/l), *M. anisopliae* (WP) (@ 2 g/l) and *Clerodendron inernte* Gaerth (@ 5%) gave a best control of grapenive mealy bug, *M. hirsutus.* The study revealed that dimethoate 30 EC @ 1.7 ml + fish oil rosin soap @ 5 g/, dimethoate 30 EC @ 1.7 ml/l were on par with each other. Dimethoate 30 EC (@ 1.7 ml/l), *V. lecanii* (WP) (@ 2g/l), *M. anisopliae* (WP) (@ 2 g/l) and *C. inerme (@* 5 %) gained a higher incremental cost benefit ratio of 58.0, 46.2, 21.5 and 21.3, respectively. Makadia *et al.* (2009) evaluated the efficiency of *V. lecanii* against *M. hirsutus* infesting custard apple. *V. lecanii* @ 2g/l water was sprayed by combining 1 ml of ranipal and teepol separately. Highest per cent mortality of 18.9 was found with *V lecanii* plus ranipal which was on par with *V. lecanii* and *V. lecanii* plus teepol (18.3 and 17.8 per cent mortality, respectively).

Banu *et al.* (2010) carried out a bioassay against *P. marginatus*. Nymphs and adults of *P. marginatus* were sprayed with the conidial suspension of *M. anisopliae*, *V. lecanii* and *B. bassiana* each at the concentration of 1×10^8 conidia/ml. About 100 per cent mortality of nymphs was recorded by all three entomopathogenic fungi, whereas, in case of adults, *V. lecanii* recorded the highest mortality of 80 per cent at seven days after treatment followed by *M. anisopliae* and *B. bassiana* with 75 and 70 per cent mortality, respectively.

Amutha and Banu (2011) worked out LD_{50} and LT_{50} of different entomopathogenic fungi on *P. solenopsis* and *P. marginatus.* Virulence of EPF was found to be decreased with the advancement of the developmental stages. LC_{50} value of *M. anisopliae* on I instars of *P. solenopsis* was $8.7x10^5$ spores/ml

but, it was $1.3x10^6$ and $5.4x10^6$ spores/ml for II instars and adults, respectively. For *B. bassiana* LC_{50} value on I instars was $9x10^5$ spores/ml whereas, it was $3.9x10^6$ and $5.3x10^7$ spores/ml on II instars and adults, respectively. Similarly, LC_{50} value for *V. lecanii* was recorded as $1.5x10^6$, $3.2x10^6$ and $1.3x10^7$ spores/ml on I instars, II instars and adults, respectively. Whereas, the LT_{50} value for different concentrations of *M. anisopliae* ranged from 3.56 to 4.50 days against I instars, 4.87 to 5.97 days against II instars and 5.66 to 6.27 days against adults. For *B. bassiana*, the LT_{50} ranged from 4.09 to 4.95 days against I instars, 5.60 to 6.10 days against II instars and 6.71 to 7.17 days against adult. In case of *V. lecanii*, the LT_{50} varied 4.79 to 5.72 days against I instars, 5.65 to 6.47 days against II instars and 7.05 to 7.22 days against adults.

In case of *P. marginatus* LC_{50} value for *M. anisopliae* was $5.0x10^5$ spores/ml on I instars, $9.8x10^5$ for II instars and $1.3x10^6$ for adults. LC_{50} value for *B. bassiana* on I instar was found to be $8.2x10^5$ spores/ml, $2.5x10^6$ spores/ml on II instars and $1.4x10^7$ spores/ml on adults. In case of *V. lecanii* the LC_{50} of $5.9x10^5$, $1.7x10^6$ and $5.01.2x10^7$ spores/ml were recorded against I instars, II instars and adults, respectively. The LT_{50} for different concentrations of *M. anisopliea* against I instar, II instar and adults ranged from 3.88 to 4.71 days, 5.19 to 6.00 days, and 6.52 to 7.02 days, respectively. For *B. bassiana*, the LT_{50} varied between 3.88 and 4.71 days against I instar, 5.19 to 6.00 days against II instar and 6.52 and 7.02 days against adults. For *V. lecanii*, the LT_{50} ranges were 4.55 to 5.46 days for I instar, 5.22 to 6.21 days for the II instar and 6.93 to 6.96 days for the adults (Amutha and Banu, 2011).

Natural enemies were the least affected by the application of biopesticides and biorational insecticides in the cotton field compared to insecticides. Spraying of chlorpyrifos and acephate proved better in bringing down the population of *P. solenopsis* with 68.60 and 72.34 per cent mortality, respectively. Out of different biopesticides, *V. lecanii* 5g/l, *B. bassiana* 5g/l, *M. anisopliae* 5g/l and bacterial symbiont of nematode (1:4) tested, *M. anisopliae* was reported to be effective with 41.42 per cent control (Kumar *et al.*, 2011). Spraying of oil based

formulation of *L. lecanii* twice at fortnight interval resulted in better control of *P. marginatus* (Banu and Gopalakrishanan, 2012).

Amutha and Banu (2015) reported that *M. anisopliae* was found promising in management of *P. marginatus* when compared with *V. lecanii* and *B. bassiana*. Difference in duration and timing of various phases of mycosis was noticed which revealed that mycosis cycle of EPF was faster in mealybug treated with *M. anisopliae* followed by *V. lecanii* and *B. bassiana*.

Ferreira *et al.* (2015) reported a potential strain of entomopathogenic nematodes against *D. brevipes* in Brazil. Mortality caused by different strains varied with temperature. At 16°C, only *Heterorhabditis indica* LPP30 and *H. baujardi* LPP35, *H. indica* CPP22, *H. indica* LPP30, *H. mexicana* Hmex and *H. bacteriophora* HP88 caused high mortality. *H. indica* LPP30 was the most effective strain against *D. brevipes*.

According to Ujjan *et al.* (2015) an isolated strain of *M. anisopliae* PDRL526, was found effective against *P. solenopsis* in Pakistan. About 6.3×10^{12} spores/acre was reported to be effective in reducing the population of adults after 13.8 and 19.6 days of spraying under screen house and field conditions, respectively. They also reported the compatibility of the strain PDRL 526 with imidacloprid which was higher (95.2 %) than other insecticides.

2.6 Variation in Total Soluble Solids (TSS) due to pest infestation

Kumar *et al.* (2003) reported the reduction in the TSS content of guava fruit due to the infestation tea mosquito bug, *Helopeltis antonii* Signeret. The TSS value varied from 13.01° Brix in healthy fruits to 9.10° Brix in fruit with 100 per cent infestation. Akoto *et al.* (2011) observed the change in the TSS in mango varieties with variation in level of fruit fly infestation. Varieties Palmer and Kent showed higher TSS with increased level infestation later reduced with time. Whereas, in case of variety Jaffna, variation in TSS showed a opposite trend where the TSS decreased with the increase in level of infestation.

Mulberry varieties like M_5 and MR_5, TSS was found to be reduced (3.7° Brix) with the infestation of leaf roller, *Diaphania pulverulentalis* Hampson (Mahadeva and Nagaveni, 2011). According to Nandre and Shukla (2013) TSS of different varieties of sapota showed the positive correlation with level of infestation of Oriental fruit fly, *Bactrocera dorsalis* (Hendel). Due to the damage caused by sucking pest in Kinnow Mandarin (*Citrus reticulate* Blanco), TSS of the fruits was found to reduce (Zubair *et al.* 2015).

Materials and methods

3. MATERIALS AND METHODS

The present study entitled "Eco-friendly management of pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae)" was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara between September 2014 and June 2016. The objectives of the investigation were to document the natural enemy fauna of *Dysmicoccus brevipes* and to formulate eco-friendly measures for the management of the mealybug. The details of the materials used and techniques adopted for conducting various experiments based on the objective set forth in this study are presented here under.

3.1 Mass culturing of pink pineapple mealybug, *Dysmicoccus brevipes*

To carry out various studies *viz.*, biology, bioassay and pot culture experiments, mealybugs were reared on pumpkin in the AINPAO laboratory, College of Horticulture, KAU, Vellanikkara. Pumpkin fruits were thoroughly washed with water and surface treated with bavistin (0.1 %). The treated pumpkins were shade dried and kept in rearing cages. Damage or wounds, if any, found on pumpkin fruits, were sealed by applying wax. Nymphs and adult female mealybugs collected from pineapple field were released on pumpkins (Plate 1).

3.2 Biology of *Dysmicoccus brevipes*

The study was conducted at ambient room conditions (temperature 31.2±1.1°C and RH of 73.31±9.66%). Twenty uniform first instar nymphs were collected from the pumpkin fruits (used in the mass culturing of mealybugs) and released on thoroughly washed and dried pineapple leaf bits of size eight centimetre length. Leaf bits were placed in Petri dish lined with wet cotton and observed daily under microscope. Duration of each instar was confirmed based on the presence of moulted exuvia of nymphs. Other parameters like adult longevity, pre-larviposition, larviposition, post-larviposition period and number of nymphs

Rearing cages

Pumpkins covered with mealybugs

Plate 1. Rearing of *Dysmicoccus brevipes* on Pumpkin

per female along with colour and shape of each stages were recorded. Morphometric observations like length and width were taken from 20 mealybugs.

3.2.1 Statistical analysis

Duration of development of all stages of *D. brevipes*, adult longevity, pre-larviposition, larviposition, post-larvipostion period and number of nymphs per female of the *D. brevipes* were recorded and expressed as mean days ± standard deviation (SD). Similarly, the morphometric parameters *viz.*, length and width (mm) were expressed as mean length and mean width ± standard deviation (SD), respectively.

3.3 Survey for collection and identification of natural enemies of *Dysmicoccus brevipes*

Purposive survey was carried out in major pineapple growing districts of Kerala *viz.*, Ernakulam, Idukki and Thrissur. Among these districts, different locations were selected for the survey, depending on the extent of pineapple cultivation (Table 1) and GPS co-ordinates of the selected location were recorded. The survey was carried out at monthly intervals from January to May, 2016. Two infested fruits as well as plants along with the mealybugs were collected from the farmer's fields and observed for the presence of natural enemies like predators, parasitoids and diseased insect.

Table 1. Locations selected for conducting survey

Districts	Locations
Ernakulam	Kaloor, Kalloorkkad, Vazhakulam, Nadukkara, Peramangalam
Idukki	Kumaramangalam, Thodupuzha
Thrissur	Vellanikkara, Kootala, Poomala

3.3.1 Predator and parasitoid of *Dysmicoccus brevipes*

Infested pineapple fruits and roots collected from the surveyed localities were examined for the presence of predators. The immature stages of predators were collected and reared to the adult stage. After the emergence of the adults, they were separated from polythene cover and preserved in alcohol (70%) and got identified. The predators got identified at the Department of Agricultural Entomology, College of Horticulture, KAU, Vellanikkara. Similar procedure was followed for the parasitoids also. After its emergence from parasitized mealybugs, the parasitoids collected were preserved in alcohol (70%). The preserved specimens were got identified from Aligarh Muslim University, Uttar Pradesh, India.

3.3.2 Isolation of entomopathogenic fungus of *Dysmicoccus brevipes*

Mealybug samples from infested fruits collected from pineapple fields were observed for the presence of dead mealybugs. The mycosed mealybug specimens were removed using a fine camel hair brush and surface sterilized with sodium hypochlorite (1%) solution for one minute and then washed three times with sterile distilled water. Then it was transferred aseptically to Petri dishes lined with moist filter paper and incubated at room temperature of (29±1°C) for two days to observe for mycelial growth, if any. Once the fungal growth was visible externally, the specimens were carefully picked up with needle and kept in Petri dish of 8.5 cm diameter containing Potato Dextrose Agar medium (PDA) (Annexure I). The Petri dishes were incubated at room temperature and examined daily for the growth of fungal mycelia.

3.3.2.1 Pathogenicity test

Pathogenicity test was carried out by spraying the spore suspension prepared from the isolated fungus on the healthy mealybug.

3.3.3 Identication of ants species associated with the *Dysmicoccus brevipes*

Ants were found associated with the mealybugs in pineapple fields under natural conditions. The ants were collected from the field and preserved in alcohol (70%). Specimens were got identified from St. Xavier's College, Aluva.

3.4 Preparation of spore concentration of entomopathogenic fungi

Cultures of the three entomopathogenic fungi *viz.*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillim lecanii* were obtained from AICRP on BCCP & W, Kerala Agricultural University, Vellanikkara were ground in wearing blender and made into liquid spore suspension. The suspension was filtered through double layered muslin cloth to remove the mycelial mat. For uniform distribution of fungal spores, 5 ml of Tween 80® (0.02%) was added to the spore suspension and filtered through a clean muslin cloth. The spore count in the fungal suspension was assessed by using a haemocytometer and was estimated using the formula suggested by Lomer and Lomer (1996).

$$\text{Number of spore/ml} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where,

X = Number of spores counted from small squares of haemocytometer

Y = Number of small squares counted in haemocytometer

400 = Total number of small squares in haemocytometer

10 = Depth factor

1000 = Conversion factor from mm^3 to cm^3

D = Dilution factor

Based on the number of spores, all the cultures were adjusted to 1×10^9 spores ml^{-1} from which the lower concentrations *viz.*, 1×10^8 and 1×10^7 were prepared by serial dilution method for bioassay studies.

3.4.1 Evaluation of entomopathogenic fungi under laboratory conditions

Spore suspensions of each of three fungi *viz.*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* at three different concentrations of (1×10^7, 1×10^8 and 1×10^9 spores ml^{-1}) were used for the bioassay (Table 2). It was carried out in the laboratory by dipping method of inoculation (Plate 2) (Mohamed, 2016). Twenty second instar nymphs of *D. brevipes* were released on pineapple leaf bits of size eight centimetre length and allowed to settle on the leaf bits to prevent them from escaping. After 24 h of releasing the nymphs, the leaf bits along with the nymphs were dipped in the 200 ml of different spore concentrations of EPF for 10 seconds. Later, the leaf bits were transferred to Petri dish (9 cm diameter) lined with moist cotton. To ensure constant humidity, all the Petri dishes containing treated nymphs were placed in a plastic container and covered with a white muslin cloth. Mortality count was taken on third, fifth and seventh day after the treatment. Cadavers of dead mealybugs were kept in humid chamber for two days to observe the hyphal growth and were observed under the microscope for mycelial growth.

3.4.2 Statistical analysis

Per cent mortality data was corrected with the control mortality by using Abbott's formula (Abbott, 1925). Per cent mortality was calculated and subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT).

Plate 2. Dipping method of inoculation

Table 2. Concentrations of entomopathogenic fungi tested under laboratory conditions

S. No	Treatments	Concentration
1	T1: *Metarhizium anisopliae*	$1x10^7$spores ml^{-1}
2	T2: *M. anisopliae*	$1x10^8$ spores ml^{-1}
3	T3: *M. anisopliae*	$1x10^9$ spores ml^{-1}
4	T4: *Beauveria bassiana*	$1x10^7$spores ml^{-1}
5	T5: *Beauveria bassiana*	$1x10^8$ spores ml^{-1}
6	T6: *Beauveria bassiana*	$1x10^9$ spores ml^{-1}
7	T7: *Lecanicillium lecanii*	$1x10^7$spores ml^{-1}
8	T8: *Lecanicillium lecanii*	$1x10^8$ spores ml^{-1}
9	T9: *Lecanicillium lecanii*	$1x10^9$ spores ml^{-1}
10	T10: Control (Distilled water)	

3.5 Evaluation of entomopathogenic fungi under pot culture experiment

To conduct pot culture experiment of Entomopathogenic fungi (EPF), pineapple (cv. Mauritius) slips were procured from Pineapple Research Station, Vellanikkara. The experiment was laid out in Completely Randomized Design (CRD) with six treatments and four replications (eight plants per replication) (Plate 3). Best concentration of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillim lecanii* were selected based on laboratory evaluation (Table 3). Nymphs of 0 to 24 h old were released at the rate of 100 nymphs per plant following the paper strip method. Paper strips were left on the pumpkins for 24 h and once the nymphs were found crawling over the stripes and settled over it, strips along with crawlers were slowly removed and kept in the plastic jar without much disturbance and later transferred to pineapple slips. One month after the release of nymphs, pre-count of mealybug numbers was taken prior to entomopathogenic fungi treatments. The treatments were applied using as pneumatic hand sprayer during the evening hours.

Table 3. Evaluation of the entomopathogenic fungi on *Dysmicoccus brevipes* in pot culture experiment

Treatments	Frequency of application
T1: *Lecanicillium lecanii* @ $1x10^8$ spores ml^{-1}	Three sprays at 10 days interval
T2: *Metarhizium anisopliae* @ $1x10^8$ spores ml^{-1}	Three sprays at 10 days interval
T3: *Beauveria bassiana* @ $1x10^8$ spores ml^{-1}	Three sprays at 10 days interval
T4: Quinalphos 25 EC @ 0.05%	Single spray
T5: Azadirachtin 1% @ 0.005%	Three sprays at 10 days interval
T6: Control	

Plate 3. Layout of pot culture experiment

3.5.1 Observation

The population of mealybugs were recorded before and after the application of each treatment by destructive sampling method. Count of the mealybugs per each plant was taken at ten days interval upto 40^{th} day after the first treatment. The dead mealybugs were collected and placed in a Petri dish lined with a moist filter paper and was observed for the mycelial growth.

3.5.2 Statistical analysis

The mean population of mealybugs of both pre and post count was analysed by Analysis of Covarience. Per cent reduction in number of mealybugs over control was analysed by ANOVA and means were separated by Duncan's Multiple Range Test (DMRT).

3.6 Estimation of Total Soluble Solids (TSS)

Twenty uniformly ripened pineapple fruits of variety Mauritius with mealybugs infestation were procured from the pineapple market at Vazhakulam. They were graded into high, medium and low level of infestation (Table 4) based on the number of mealybugs present per fruit (Plate 4). Subsequently, the fruits were peeled and portions of fruit were cut from top, middle and bottom. A drop of fruit juice was squeezed out on the refractometer from each of the cut portion for testing TSS value.

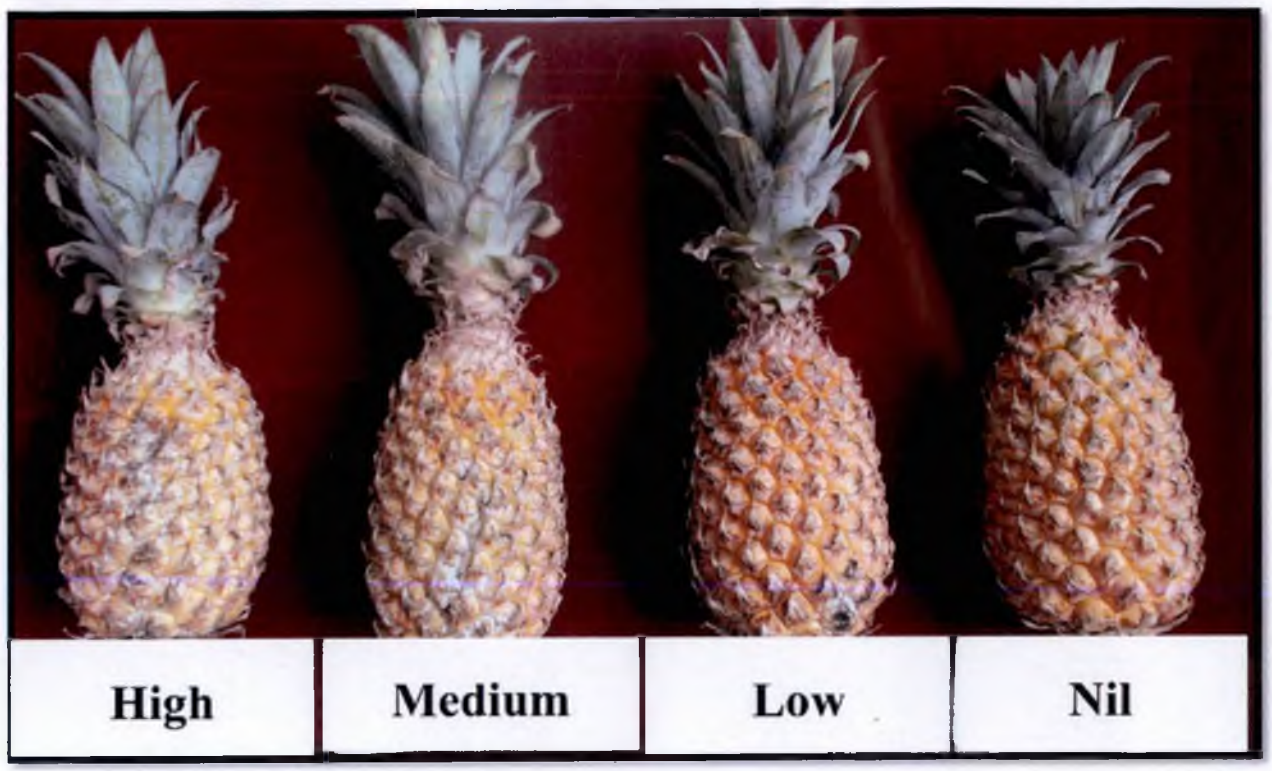


Plate 4. Pineapple fruits with different levels of mealybug infestation

Table 4. Classification for pineapple fruits based on the different level mealybugs infestation

Level of infestation	No. of mealybugs per fruits
High	>251
Medium	151-250
Low	51-150
Nil	Zero

3.6.1 Statistical analysis

Change in TSS was calculated and subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT).

Results

4. RESULTS

The results obtained from the study "Eco-friendly management of pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae)" are presented in this chapter.

4.1 Biology of *Dysmicoccus brevipes*

The biology of *D. brevipes* was studied in the laboratory of All India Network Project on Agricultural Ornithology, College of Horticulture, KAU, Vellanikkara. Twenty of first instar crawlers of same age were used for the study. The life-cycle of *D. brevipes* showed the presence of three nymphal instars and an adult stage (Table 5). In the present study males were absent and females were found to reproduce through parthenogenesis.

4.1.1 Nymphal instars

Three nymphal instars were present in the life cycle of *D. brevipes*. Differences in body shapes and dimensions of each instar were used for distinguishing the different stages. The duration of each instar was recorded by observing the presence of moulting exuvia. The nymphal period ranged from 38 to 42 days with an average of 40.2±1.10 days (Table 5).

4.1.1.1 First instar nymph

The first instar nymphs were active and fast moving. They had dorso-ventrally flattened body with pinkish colour (Plate 5). Antennae consisted of six segments (Plate 6). Body of the crawlers were partially covered with waxy coating and they were found aggregating beneath the adult female's body. The duration of the first instar nymph ranged from 10 to 12 days with an average of 10.8±0.67 days (Table 5). The length and width of crawlers varied from 0.38 to 0.48 mm and 0.24 to 0.25 mm (mean length 0.43±0.02 mm and mean width 0.24±0.01 mm), respectively (Table 6).

Table 5. Biology of *Dysmicoccus brevipes*

Parameter	Mean*duration ± S.D (days)	Duration (days)
First instar	10.8±0.67	10-12
Second instar	13.7±0.65	13-15
Third instar	15.6±0.58	15-17
Nymphal period	40.2±1.10	38-42
Pre-larviposition period	8.7±0.78	8-10
Larviposition period	4.5±0.51	4-5
Post-larviposition period	9.8±1.16	7-10
Adult female	23.2±0.78	22-24
Total life cycle	63.4±1.50	61-66
No. of nymphs per adult	**Mean*(no. of nymph) ± S.D**	**Total number of nymphs**
	144.5±15.1	107-154

*Mean of 20 observations

a. First instar nymph

b. Second instar nymphs

c. Third instar nymph

d. Adult female

Plate 5. Developmental stages of *Dysmicoccus brevipes*

4.1.1.2 Second instar nymph

Second instar nymphs were similar to the first instar nymphs having pinkish flattened body (Plate 5) and six segmented antennae (Plate 6). Duration of the crawlers lasted for 13 to 15 days with an average of 13.7±0.65 days (Table 5). The length of the nymphs ranged from 0.56 to 0.78 mm with an average of 0.67±0.07 mm and width varied from 0.29 to 0.38 mm with a mean of 0.34±0.03 mm (Table 6).

4.1.1.3 Third instar nymph

Nymphs were pinkish with flattened body in the third instar. Body of the nymphs were covered with waxy coating (Plate 5). Contrary to the first two instars, the third instar nymph had seven segmented antenna (Plate 6). Seventeen pairs of waxy filaments were present on the ventral side of the body. The duration of the third instar nymphs ranged between 15 to 17 days with a mean of 15.6±0.58 days (Table 5). The size of the nymphs varied from 1.27 to 1.46 mm length and 0.71 to 0.89 mm width with an average length of 1.05±0.10 mm and width 0.78±0.07 mm (Table 6).

4.1.4 Adult female

Adults had a convex body with pinkish colour. Body of the adults were covered with thick layer of waxy coating (Plate 5). Antennae were long with eight segments (Plate 6). Adults also had seventeen pairs of waxy filaments as in case of third instar nymphs. Adults lived for about 22 to 24 days with a mean longevity of 23.2±0.78 days (Table 5). The average size of the adults was 1.78±0.21 mm length and 1.15±0.02 mm width with the length varied from 1.72 to 1.98 mm while width 0.93 to 1.33 mm (Table 6).

Adults were ovoviviparous and reproduced parthenogenetically. Adult mealybugs secreted a loose waxy filament at the posterior end of the body. The nymphs were found within this waxy material. Adults retained their progeny

a. First instar (40x)

b. Second instar (40x)

c. Third instar (40x)

d. Adult female (40x)

Plate 6. Antennal segments in different instars of *Dysmicoccus brevipes*

underneath the body till the crawlers moulted in to second instar. The total duration required to complete a life cycle of *D. brevipes* ranged from 61 to 66 with an average duration of 63.4±1.50 days.

4.1.5 Pre-larviposition period, larviposition period, post-larviposition and number of nymphs

The pre-larviposition period of the females lasted for about eight to ten days with an average of 8.75±0.78 days. The duration of larviposition varied from four to five days with an average of 4.5±0.51 days, while the post-larviposition period ranged from seven to ten days with an average of 9.8±1.16 days. Number of crawlers emerged from each adult ranged from 107 to 154 with a mean value of 144.52±15.1 (Table 5).

Table 6. Morphometrics of life stages of *Dysmicoccus brevipes*

S. No.	Stage	Mean* length (mm)	Range (mm)	Mean* width (mm)	Range (mm)
1.	First instar	0.43±0.02	0.38-0.48	0.24±0.01	0.24-0.25
2.	Second instar	0.67±0.07	0.56-0.78	0.34±0.03	0.29-0.38
3.	Third instar	1.05±0.10	1.27-1.46	0.78±0.07	0.71-0.89
4.	Adult	1.78±0.21	1.72-1.98	1.15±0.02	0.93-1.33

*Mean of 20 observations

4.2 Survey and documentation of natural enemies of *Dysmicoccus brevipes*

Samples collected from different locations of Ernakulam, Idukki and Thrissur consisted of four species of predators, a parasitoid and a fungus. Predators included *Cacoxenus perspicax* (Knab) (Drosophilidae: Diptera), *Spalgis epius* (Westwood) (Lycaenidae: Lepidoptera), two species of *Scymnus* (Coccinellidae: Coleoptera), parasitoid, *Chartocerus* sp. (Signiphoridae: Hymenoptera) and the fungus *Apergillus* sp. (Table 7).

Apart from the *Scymnus* sp. another coccinellid predator, *Pharoscymnus horni* Weise was also reported from Vellanikkara. It was observed to be feeding on the scale insects, *Diaspis bromeliae* (Kerner) (Family: Diaspididae) infesting on the pineapple plants (Plate 7 to 12).

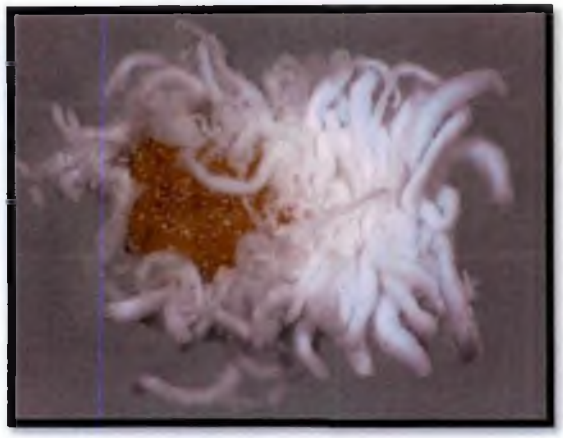
4.2.1 Relative abundance of the predators and parasitoids in different locations

Among different natural enemies collected from selected locations of Ernakulam, Iduki and Thrisur districts, *Scymnus* sp. was found to be the most abundant in all the six locations and it accounted for 68.75 per cent of the total natural enemies reported from all the locations (Table 8). It was followed by *Spalgis epius,* which was reported in larger number only from three locations *viz.,* Kalloorkkad, Thodupuzha and Vellanikkara with the occurrence of 48.39, 29.03 and 22.58 per cent, respectively. Similarly, *Cacoxenus perspicax* was also collected from three locations which includes Nadukkara (8.69%), Thodupuzha (10.34%) and Vellanikkara (22.22%). during the survey while, few number of parasitoid, *Chartocerus* sp. was observed and was reported from only in Vazhakulam and Vellanikkara.

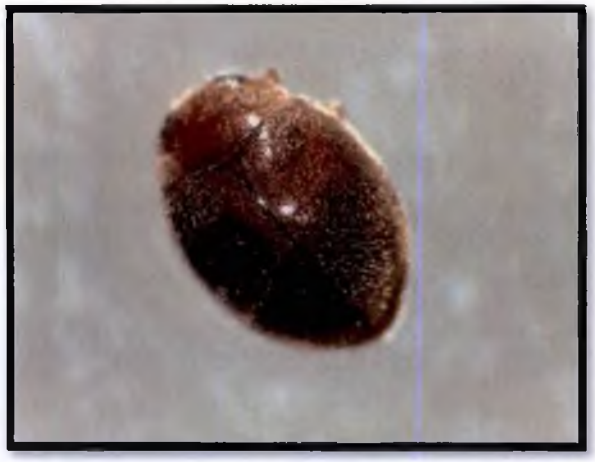
4.2.2 Fungus isolated from the infected mealybugs during the survey

Aspergillus sp. infection was noticed on *D. brevipes* in Kalloorkkad and Vellanikkara. It appeared to be a chance infection. However, under laboratory

a. Grub (35x)



b. Pupa (35x)



c. Adult

Plate 7 (i). Developmental stages of *Scymnus* sp. 1

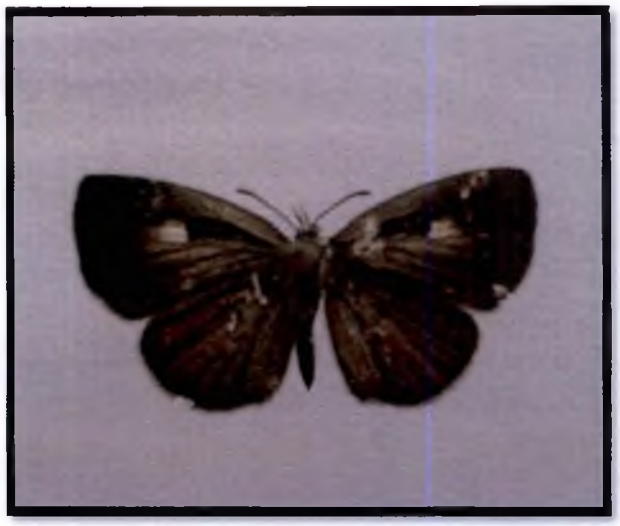
a. Pupa (35x)

b. Adult

Plate 7 (ii). Developmental stages of *Scymnus* sp. 2

a. Larva

b. Pupa



c. Adult

Plate 8. Developmental stages of *Spalgis epius*

a. Pupa (35x)

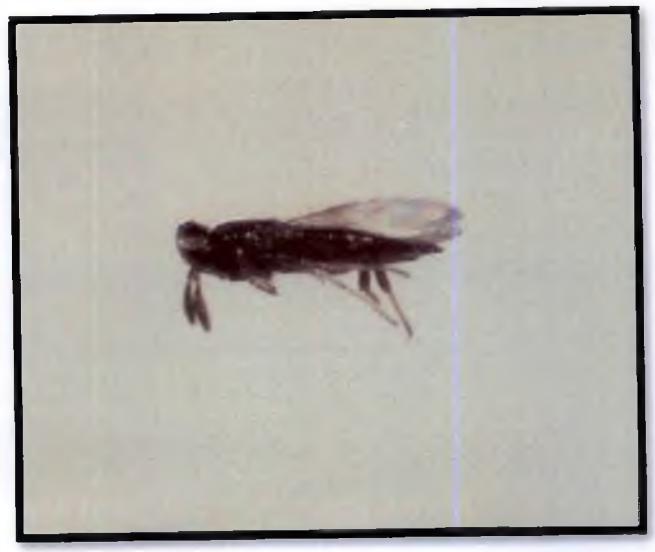
b. Adult (35x)

Plate 9. Developmental stages of *Cacoxenus perspicax*

a. Grub (35x)

b. Adult (35x)

Plate 10. Developmental stages of *Pharoscymnus horni*



Adult (35x)

Plate 11. Parasitoid – *Chartocerus* sp.

a. Diseased mealybugs on pineapple fruit

b. Mycelial growth on mealybug (35x)

c. Colony of *Aspergillus* sp.

Plate 12 Mealybugs infected with *Aspergillus* sp.

Camponatus mitis (35x)

Technomyrmex albipes (35x)

Plate 13. Ant species associated with Dysmicoccus brevipes

condition the isolated *Aspergillus* sp. failed to cause infection when sprayed the same on the mealybugs.

4.2.4 Ants associated with *Dysmicoccus brevipes*

Two species of ants, *Camponotus mitis* (Smith) (Formicidae: Formicinae) and *Technomyrmex albipes* (Smith) (Formicidae: Dolichoderinae) were found associated with *D. brevipes* in the pineapple fields (Plate 13). These ants were collected from the mealybug infested pineapple plants of Nadukkara (Ernakulam district) and found tending the mealy bugs below the ground.

Table 7. Distribution of natural enemies in selected locations of Ernakulam, Idukki and Thrissur districts

Districts	Location	GPS Co-ordinates	Natural enemies														
			January 2016			February 2016			March 2016			April 2016			May 2016		
			Pr	Pa	F	Pr	Pa	F	Pr	Pa	F	Pr	Pa	F	Pr	Pa	F
Ernakulam	Kaloor	9°59′49.524′′N 76°18′10.134′′E	Sc.	-	-	Sc	-	-	Sc	-	-	Sc	-	-	Sc	-	-
	Kalloorkkad	9°58′11.744′′N 76°40′18.743′′E	Sc, Sp	-	As	Sc, Sp	-	-	Sc	-	-	Sc, Sp	-	-	Sc, Sp	-	-
	Peramangalam	10°34′24.848′′N 76°10′10.844′′E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Nadukkara	9°56′26.056′′N 76°36′54.997′′E	Sc	-	-	Sc	-	-	Sc	-	-	Sc, C	-	-	Sc	-	-
	Vazhakulam	9°56′49.049′′N 76°36′9.241′′E	-	-	-	-	-	-	Sc	-	-	Sc	-	-	Sc	Ch	-
Idukki	Kumaramangalam	9°56′30.887′′N 76°42′58.810′′E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Thodupuzha	9°53′34.728′′N 76°43′19.589′′E	Sc	-	-	Sc	-	-	Sc	-	-	Sc, Sp	-	-	Sc, C	-	-
Thrissur	Kootala	18°27′38.290′′N 73°54′50.119′′E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Poomala	10°36′34.438′′N 76°14′2.558′′E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Vellanikkara (PRS)	10°32′42.770′′N 76°16′26.324′′E	Sc, Sp, C	Ch	As	Sc, Sp, C	-	As	Sc, C,		-	-	-	-	-	-	-

Pr-Predator, Pa-parasitoids, F-Fungus, Sc-*Scymnus* sp., Sp- *Spalgis epius*, C- *Cacoxenus perspicax*, Ch- *Chartocerus* sp., A- *Aspergillus sp.*
GPS- *Global Positioning System*

Table 8. Relative abundance of predators and parasitoid of *Dysmicoccus brevipes* in selected locations of Ernakulam, Idukki and Thrissur districts

Location	Number of natural enemies	Number of predators			Number of parasitoid	Relative abundance of predators			Relative abundance of parasitoid
		Scymnus sp.	*Spalgius epius*	*Cacoxenus perspicax*	*Chartocerus* sp.	*Scymnus* sp.	*Spalgius epius*	*Cacoxenus perspicax*	*Chartocerus* sp.
Kaloor	31	26	-	-	-	83.87	-	-	-
Kalloorkkad	35	25	15	-	-	71.42	48.39	-	-
Nadukkara	23	18	-	2	-	78.26	-	8.69	-
Vazhakulam	22	14	-	-	6	63.63	-	-	27.27
Thodupuzha	29	20	9	3	-	68.96	29.03	10.34	-
Vellanikkara	36	18	7	8	5	50	22.58	22.22	13.88
Total	176	121	31	13	11	68.75	17.61	7.39	6.25

4.3 Evaluation of entomopathogenic fungi on *Dysmicoccus brevipes* under laboratory conditions

To determine the efficacy of entomopathogenic fungi under laboratory, different concentrations ($1x10^7$, $1x10^8$ and $1x10^9$ spores ml^{-1}) of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* were prepared. Second instar nymphs of *D. brevipes* (20) were used for the study. Observations on mortality were recorded on third, fifth and seventh day after the treatment and per cent mortality was worked out by counting the dead mycosed nymphs.

Nymphs infected with the entomopathogenic fungus were hard and mummified. After 24 h of the death of the nymphs, germination of the conidia and penetration of hyphae through the integuments of nymphs were observed. Later, entire body surface was covered with mycelial growth (Table 9).

After three days of treatment application, significant difference was observed in the mortality of the nymphs in all the treatments (Table 10). The highest mortality of 15 per cent was observed in *L. lecanii* @ $1x10^9$ spores ml^{-1} while, a mortality of 11.67 and 13.33 per cent was recorded at $1x10^7$ and $1x10^8$ spores ml^{-1}, respectively. This was followed by *B. bassiana* where mortality of varied from 11.67 per cent at $1x10^7$ spores ml^{-1} to 13.33 per cent at $1x10^9$ spores ml^{-1}. However, *M. anisopliae* $1x10^9$ spores ml^{-1} resulted in least mortality of 8.33 per cent followed by 6.67 per cent mortality was observed at $1x10^7$ and $1x10^8$ spores ml^{-1}.

Similar trend was observed after five days after treatment application, where *L. lecanii* treatment caused mortality of 36.67, 33.33 and 26.67 per cent was observed at $1x10^9$, $1x10^8$ and $1x10^7$ spores ml^{-1}, respectively. This was followed by *B. bassiana* where $1x10^9$ spores ml^{-1} gave a mortality of 31.67 per cent while, both $1x10^8$ and $1x10^7$ spores ml^{-1} concentrations of *B. bassiana* recorded mortality of 28.33 per cent, respectively. Mortality recorded in *M. anisopliae* varied from 15 per cent at $1x10^7$ spores ml^{-1} to 20 per cent at $1x10^9$ spores ml^{-1} (Table 10).

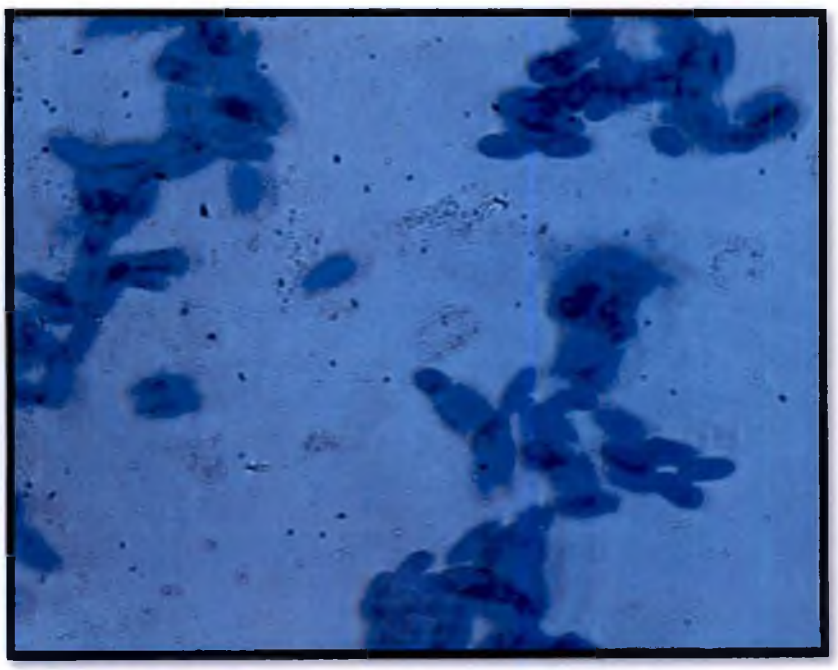
On seventh day after treatment highest mortality was observed in *L. lecanii* treatment @ $1x10^9$ spores ml^{-1} (66.67%) followed by 56.67 per cent at $1x10^8$ spores ml^{-1} and 43.33 per cent at $1x10^7$ spores ml^{-1}. Similarly, in *B. bassiana* treatment, a maximum reduction in nymphs was observed with 60 per cent at 10^9 spores ml^{-1} which was on par with the mortality recorded by *L. lecanii* @ 10^9 spores ml^{-1}. About 53.33 and 46.67 per cent reduction of nymphs was noticed at $1x10^8$ and $1x10^7$ spores ml^{-1} of *B. bassiana*, respectively. Least mortality of the nymphs was recorded with *M. anisopliae* treatment where 40 per cent mortality was recorded at $1x10^9$ spores ml^{-1} and 33.33 and 26.67 per cent mortality at $1x10^8$ and $1x10^7$ spores ml^{-1}, respectively (Table 10).

Among the three different entomopathogenic fungi treated, maximum mortality of nymphs was recorded in *L. lecanii* $1x10^9$ spores ml^{-1} which was on par with the *B. bassiana* $1x10^9$ spores ml^{-1}. Least of mortality was recorded in *M. anisopliae* treatment (40%) at $1x10^9$ spores ml^{-1} and was significantly different with mortality recorded in *L. lecanii* and *B. bassiana* at $1x10^9$ spores ml^{-1} treatments. The mortality recorded by higher spore concentrations of the three entomopathogenenic was found to be statistically on par with the succeeding lower spore concentrations. Hence, $1x10^8$ spores ml^{-1} was used to test the efficacy with potculture.

Table 9. Symptoms and conidial characteristics of entomopathogenic fungi

Entomopathogenic fungi	Infection symptoms	Microscopic characteristics
Metarhizium anisopliae	Initially nymphs were covered with white mycelial growth. Later white mycelia were replaced by green coloured spores. Due to this green coloured powdery mass was observed in the final stage (Plate 14)	Conidiophores were hyaline and short, bearing parallel oriented conidiogenous cells. Conidia were green in colour, single celled and ovoid to cylindrical shaped
Beauveria bassiana	Body of the nymphs was fully covered with white conidial spores (Plate 15)	Conidiophores were single or branched, individual conidiophores with a cluster of short and ovoid conidiogenous cells; conidia were single, smooth and round shaped
Lecanicillium Lecanii	Dead nymphs were entirely covered with the white coloured mycelial growth (Plate 16)	Hyphae septate and hyaline; conidiophores hyaline, well differentiated and erect. Whereas the conidia round to be ovoid and slightly hyaline

White mycelial growth with greenish powdery mass of *Metarhizium anisopliae* (35x)



Conidia of *Metarhizium anisopliae* (100x)

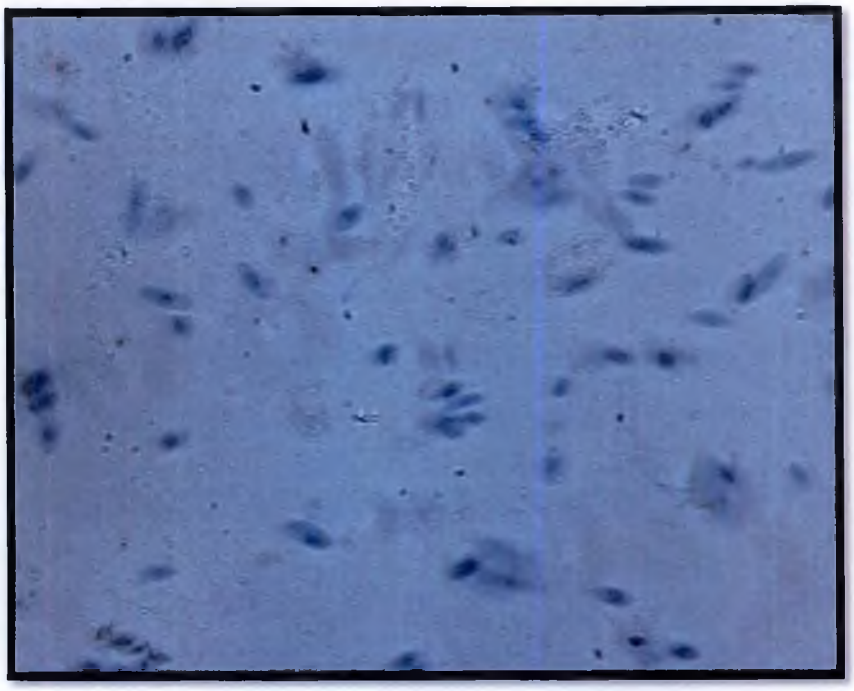
Plate 14. Infection symptoms and conidial characteristics of *Metarhizium anisopliae*

Whitish powdery growth on mealybug of *Beauveria bassiana* (35x)

Conidia of *Beauveria bassiana* (100x)

Plate 15. Infection symptoms and conidial characteristics of *Beauveria bassiana*

a. White mycelial growth on mealybug of *Lecanicillium lecanii* (35x)



Conidia of *Lecanicillium lecanii* (100x)

Plate 16. Infection symptoms and conidial characteristics of *Lecanicillium lecanii*

Table 10. Effects of entomopathogenic fungi on *Dysmicoccus brevipes* under laboratory conditions

Treatments	Mortality of nymphs of *D. brevipes* (%)		
	3 DAT	5 DAT	7 DAT
T1: *M. anisopliae* (10^7 spores ml^{-1})	6.67^{cd} (14.76)	15.00^{d} (22.29)	26.67^{e} (30.78)
T2: *M. anisopliae* (10^8 spores ml^{-1})	6.67^{cd} (14.79)	16.67^{cd} (24.05)	33.33^{de} (35.29)
T3: *M. anisopliae* (10^9 spores ml^{-1})	8.33^{bc} (16.59)	20.00^{bcd} (26.07)	40.00^{cde} (39.15)
T4: *B. bassiana* (10^7 spores ml^{-1})	11.67^{abc} (19.88)	28.33^{ab} (32.02)	46.67^{bcd} (43.08)
T5: *B. bassiana* (10^8 spores ml^{-1})	10.00^{abc} (18.05)	28.33^{abc} (32.02)	53.33^{abc} (47.01)
T6: *B. bassiana* (10^9 spores ml^{-1})	13.33^{ab} (21.34)	31.67^{a} (34.16)	60.00^{ab} (50.86)
T7: *L. lecanii* (10^7 spores ml^{-1})	11.67^{abc} (19.88)	26.67^{abc} (31.07)	43.33^{bcde} (41.15)
T8: *L. lecanii* (10^8 spores ml^{-1})	13.33^{ab} (21.14)	33.33^{a} (35.17)	56.67^{abc} (48.93)
T9: *L. lecanii* (10^9 spores ml^{-1})	15.00^{a} (22.79)	36.67^{a} (37.23)	66.67^{a} (54.78)
T10: Control (Distilled water)	1.67^{d} (4.73)	3.33^{d} (8.83)	3.33^{f} (8.83)
CD(0.05)	5.818	11.63	17.38

Mean values in columns followed by same alphabet(s) are not significantly different by DMRT at P=0.05

DAT- Days After Treatment

Figures in the parentheses are arcsin transformed values

Values in the columns are mean of three replications

4.4 Evaluation of entomopathogenic fungi (EPF) under pot culture experiment

Efficacy of entomopathogenic fungi was evaluated under pot culture experiment. The concentration of the EPF was taken based on laboratory evaluation. One month after the release of the first instar nymphs on pineapple plants, pre count was taken a day before the treatments application. *L. lecanii*, *M. anisopliae* and *B. bassiana* @ $1x10^8$ spores ml^{-1} and azadirachtin (1% @ 0.005%) were sprayed three times by maintaining a standard check (quinalphos 25EC @ 0.05% - single spray). Each treatment was repeated at ten days intervals and the observation was taken after each spray up to 40 days of the first application by destructive sampling method.

The mean pre and post-treatment counts of mealybugs was taken at ten days after the treatment application. The pre-treatment count of the mealybug found to be non significant (Table 11). Ten days after the application of quinalphos there was a drastic reduction in the number of mealybugs with the reduction of 96.73 per cent followed by azadirachtin which resulted in the reduction of 87.75 per cent of the mealybugs and statistically this was on par with the quinalphos (Table 12). Among three different entomopathogenic fungi *viz., L. lecanii, B. bassiana* and *M. anisopliae* treated, *M. anisopliae* effected maximum reduction of 59.29 per cent, followed by *B. bassiana* (30.13 %) and *L. lecanii* (17.26 %). Effects of all the entomopathogenic fungus in the reduction of mealybugs was found on par with each other.

Twenty days after the treatment, *M. anisopliae* and *B. bassiana* treatments had similar effect in lowering the number of mealybugs with a reduction of 72.72 and 70.98 per cent, respectively, while in *L. lecanii* could reduce to 56.37 per cent. However, all the treatments were on par with each other. Compared to the entomopathogenic fungi, quinalphos and azadirachtin treatments reduced the mealybug population significantly (96.47 and 94.43% reduction, respectively).

At 30 DAT, *L. lecanii* treatment was more effective in reducing the mealybugs (90.04%) and it was on par with the quinalphos treatment (95.20 %). The effect of *B. bassiana* (76.04 %), azadirachtin (75.23%) and *M. anisopliae* (74.36%), were on par with each other. The efficacy of all the entomopathogenic was found to be increased with repeated sprays and also time lapse after the treatment.

Table 11. Effect of entomopathogenic fungi on *Dysmicoccus brevipes* in the pot culture experiment.

Treatments	PTC	Mean population at 10 DAT		
		†† First spray	†† Second spray	†† Third spray
T1- *L. lecanii* @ $1x10^8$ spores ml^{-1} (three sprays at ten days interval)	87.25 (9.17)	46.73 (6.59)	29.35 (5.37)	7.52 (3.04)
T2- *M. anisopliae* @ $1x10^8$ spores ml^{-1} (three sprays at ten days interval)	58.75 (7.52)	27.76 (5.23)	24.46 (4.69)	23.50 (4.79)
T3- *B. bassiana* @ $1x10^8$ spores ml^{-1} (three sprays at ten days interval)	64.50 (7.859)	45.37 (6.75)	19.39 (4.45)	22.13 (4.65)
T4- Quinalphos 25EC @ 0.05% (single spray)	66.75 (8.18)	*0.89 (1.44)	*12.64 (2.58)	*7.57 (1.85)
T5- Azadirachtin 1% @ 0.005% (three sprays at ten days interval)	38.25 (6.20)	14.67 (3.51)	12.33 (2.75)	24.36 (4.28)
Control	57.12 (7.57)	68.44 (8.27)	69.45 (8.19)	81.92 (9.85)
CD (0.05)	NS	22.76 (1.58)	21.38 (NS)	28.42 (1.89)

Values in the columns are mean of four replications

Figures in the parenthesis are square root transformed values (x+0.5)

PTC- Pre-treatment Count, DAT- Days After Treatment

*- Single spray treatment

††- Values in the parenthesis are adjusted means of square root transformed values based on ANOCOVA

Table 12. Per cent reduction of *Dysmicoccus brevipes* over control by entomopathogenic fungi in pot culture experiment

Treatments	Per cent reduction over control		
	First spray	Second spray	Third spray
T1- *L. lecanii* @ $1x10^8$ spores ml^{-1} (three sprays at ten days interval)	17.26^{d}	56.37^{c}	90.04^{a}
T2- *M. anisopliae* @ $1x10^8$ spores ml^{-1} (three sprays at ten days interval)	59.29^{bc}	72.72^{b}	74.36^{b}
T3- *B. bassiana* @ $1x10^8$ spores ml^{-1} (three sprays at ten days interval)	30.13^{cd}	70.98^{bc}	76.04^{b}
T4- Quinalphos 25EC @0.05 % (single spray)	$*96.73^{a}$	$*96.47^{a}$	$*95.20^{a}$
T5- Azadirachtin 1% @ 0.005% (three sprays at ten days interval)	87.75^{ab}	94.44^{a}	75.23^{b}
CD (0.05)	34.19	16.17	12.59

Means in columns followed by same alphabet(s) is not different by DMRT at P=0.05

DAT- Days After Treatment, *- Result of single spray treatment

Values in the columns are mean of four replications

4.5 Estimation of Total Soluble Solids (TSS)

Quantification of TSS in the graded pineapple fruits showed a significance differences in TSS with different levels of mealybug infestation. Heavy infestation of mealybugs caused a reduction in TSS content. TSS of 8.74° Brix was recorded in the fruits with higher level of infestation of mealy bugs. Whereas, in the fruits with medium level of mealybugs infestation the TSS was 9.94° Brix. In case of fruits with low level of infestation of mealybugs the TSS was 11.30 Brix which was on par with the TSS of the fruits (11.38° Brix) without mealybugs infestation (Table 13).

Table 13. Effect of *Dysmicoccus brevipes* infestation on TSS in pineapple fruits

Class	TSS (° Brix)
High	8.74[c]
Medium	9.93[b]
Low	11.31[a]
Zero	11.38[a]
CD (0.05)	0.258

Means in columns followed by same alphabet(s) are significantly not different by DMRT at P=0.05

Values in the columns are mean of five replications

Discussion

5. DISCUSSION

Results of the work entitled "Eco-friendly management of pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae)" with the objectives to document the natural enemy fauna of *D. brevipes* and to formulate eco-friendly measure for the management of pest population are discussed here under with the following headings.

1. Biology of *Dysmicoccus brevipes*
2. Survey and documentation of natural enemies of *Dysmicoccus brevipes*
3. Evaluation of entomopathogenic fungi under laboratory conditions
4. Evaluation of entomopathogenic fungi under pot culture experiment
5. Impact of infestation on fruit quality

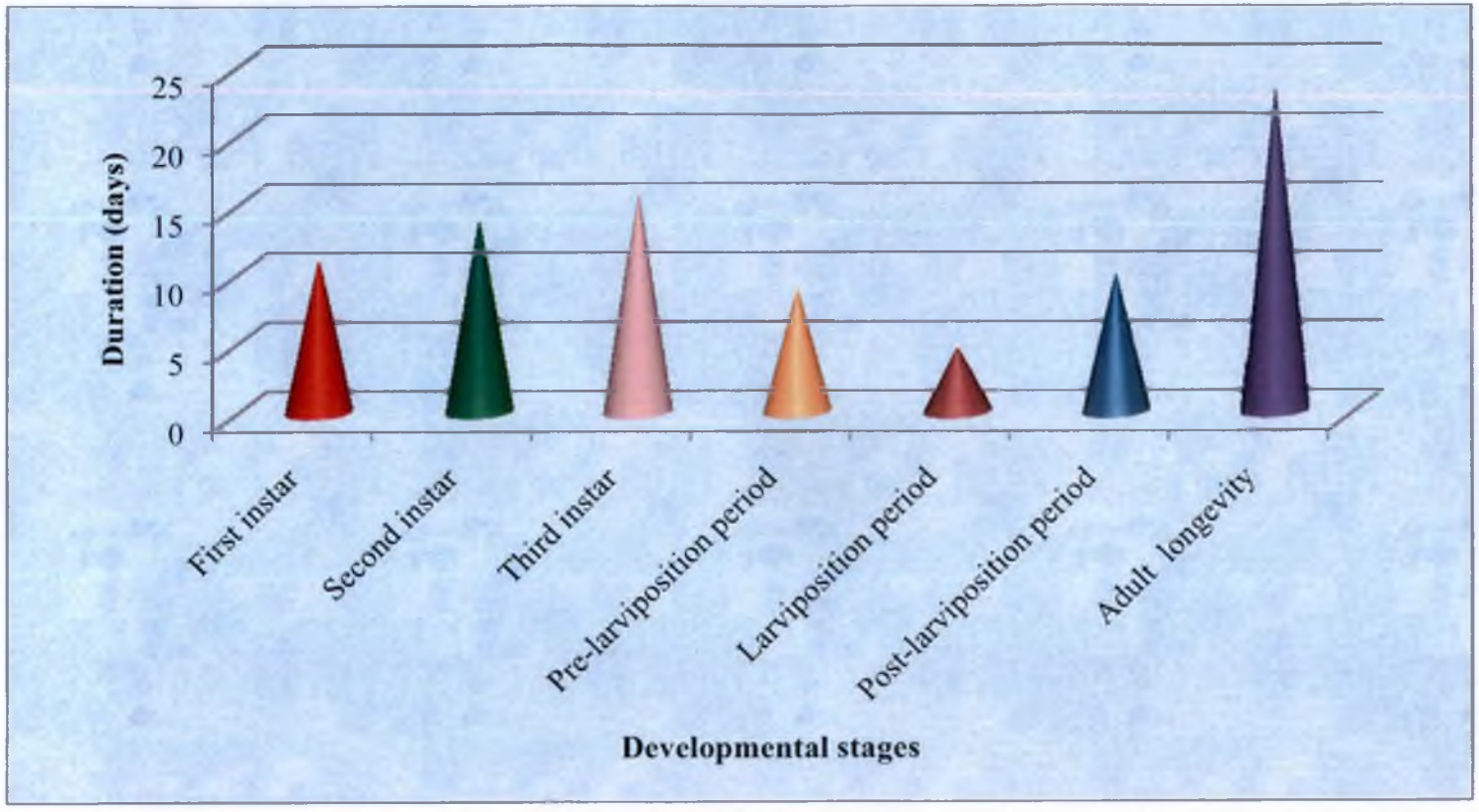
5.1 Biology of *Dysmicoccus brevipes*

Laboratory studies on biology of *Dysmicoccus brevipes* was conducted on pineapple leaf bits. The life-cycle of *D. brevipes* comprised of three nymphal instar and adult stage only with the females, probably by parthenogenetic mode of reproduction.

5.1.1 Life stages of *Dysmicoccus brevipes*

In total, three nymphal instars were observed in the life cycle of *D. brevipes* (Fig. 1). Variation in the morphometric observation was found among each instars. The duration between each instar arrived based on the occurrence of moulting exuvia revealed an average nymphal period of 40.2±1.10 which ranged from 38 to 42 days (Table 5) as reported by Bertin *et al.* (2013) where three nymphal instars of *D. brevipes* when reared on leaves of grape (*cv.* Italia) had period of 42.20±1.07 days.

nental stages of *Dysmicoccus brevipes*



5.1.2 First instar nymph of *D. brevipes*

The first instar nymphs were dorsoventrally flattened and pinkish in colour. They were found aggregating beneath the female's body. Similar observation was recorded by Ullah *et al.* (1993), while studying the life-cycle of *D. brevipes* on the guava leaves. The average duration of first instar nymphs lasted for 10.85±0.67 days which varied from 10 to 12 days (Table 5). However, longer duration of 14 days was observed by Ito (1938), when reared on pineapple leaf bits with the average temperature of 74.4° F. Bertin *et al.* (2013) also observed longer duration of first instar (13.46± 0.48 days) of *D. brevipes* studied on the leaves of the grape (cv. Italia) which was contrast to the present finding. Variation in the duration of instar nymphs might be due to the difference in the nutritional quality of the host used besides varied temperature range and the poor nutritional quality might have affected developmental period of nymphs.

The mean length of the first instars was 0.43±0.02 mm which varied from 0.38 to 0.47 mm and width 0.24±0.01 mm which ranged from 0.24 to 0.25 mm. Antennae had six segments. The present observations were in accordance with the mean length of 0.55 mm and mean width of 0.27 mm of *D. brevipes* as reported by Ghosh and Ghosh (1984).

5.1.3 Second instar nymph of *Dysmicoccus brevipes*

Second instar nymphs were almost similar to that of first instar nymphs in shape, colour and number of antennal segments except in their body size. Duration of the second instars lasted for 13 to 15 days with an average of 13.7±0.65 days (Table 5). The present finding contradicted the observations of Ito (1938) where the second instar nymphal period got over in 9.8 days. However, the result of Bertin *et al.* (2016) was in conformity to the present study where the mean duration of 13.72±0.54 days was noticed when *D. brevipes* was reared on the leaves of grape (cv. Niagara Rosada).

The length of the second instar nymphs ranged from 0.56 to 0.785 mm with an average of 0.67±0.07 mm, while width varied from 0.29 to 0.38 mm with a mean of 0.34±0.03 mm (Table 6). Antennae had six segments in the second instar also. This finding was in conformity to the study of Ghose and Ghose (1984) revealing of 0.7 mm length and 0.33 mm width of *D. brevipes*.

5.1.4 Third instar nymph of *Dysmicoccus brevipes*

Body of the third instar nymph was covered with wax coating. The duration of the third instar lasted for 15 to 17 days with an average of 15.6±0.58 days (Table 5). On the contrary, an increased duration of the third instar of *D. brevipes* was reported (17.97±0.79 days) on leaves of grape (cv. Italia) as by Bertin *et al.* (2016).

Length of the third instar nymph varied from 1.27 to 1.46 mm with an average of 1.05±0.10 mm and width ranged from 0.71 to 0.89 mm with a mean of 0.78±0.07 mm (Table 6). They had seven segmented antennae which was different from the earlier instars. These findings were similar to the study of Ghose and Ghose (1984) where the mean body length of 1.15 mm and width of 0.69 mm were observed in *D. brevipes*.

5.1.5 Adult

Adults represented only by females being pinkish with convex body covered with a thick waxy coating are in conformity with Ito (1938) who reported the absence of males in the parthenogenic form. Similarly, Ghose (1976) observed the absence of males among *D. brevipes* reared on sprouted potatoes. On the contrary, Lim (1973) reported presence of males in the bisexual race of *D. brevipes* on pineapple in West Malaysia.

Adult females lived for about 22 to 24 days with a mean life span of 23.2±0.78 days (Table 5). However, the maximum adult longevity of 55 days (Ito, 1938) and 17 to 49 days (Lim, 1973) was reported in *D. brevipes*. Mean length of

the adults of *D. brevipes* was 1.78±0.21mm and width of 1.15±0.11 mm with eight segmented antennae. Length of the adult female was almost similar to the observations of Ghose and Ghose (1984) where the adult of *D. brevipes* measured about 1.37 mm in length and width of 0.81 mm.

A layer of loose waxy filaments was produced from the posterior end of the female body. This waxy material enclosed the first instar nymphs. In adults, ovoviviparous type of reproduction was observed. Adults retained their progeny underneath the body till the nymphs moulted into second instar. The present observations were in conformity with the findings of Ito (1938) and Lim (1976) where adults of *D. brevipes* were ovoviviparous and they observed the production of nymphs by the female adults.

The total life cycle of was completed within 61 to 66 days with an average of 63.4±1.5 days. On contrast, Ito (1938) observed the prolongrd life cycle of 90 days in *D. brevipes* which might be due to varied abiotic factors and host plant.

5.1.7 Pre-larviposition period, larviposition period, post-larviposition period and number of nymphs

In females, the pre-larviposition period lasted for about 8 to 10 days with a mean of 8.75±0.78 day, while larviposition period from four to five days with an average of 4.5±0.51 days and post-larviposition period from seven to ten days with a mean of 9.8±1.16 days (Table 5). However, extended period of pre-larviposition and larviposition of 27 and 25 days, respectively and post-larviposition period of five was reported by Ito (1936). Lim (1973) also observed longer duration of pre-larviposition (14.6 days) and larviposition period (9.1 days). In the present study, mean number of nymphs produced was 144.5±15.1 ranging from 107 to 154 crawlers per female.

However, Lim (1973) observed that the gravid female of *D. brevipes* produced 19 to 137 crawlers under laboratory conditions. In contrast to the present findings, Bertin *et al.* (2016) observed the production of 30.86±2.74 and

24.23±2.74 nymphs per female reared on the leaves of the grape cultivars, Italia and Niagara Rosada, respectively. The difference in the reproduction rate might be due to the presence of variation in nutrient contents in the leaves of the cultivar, Niagara Rosada when compared with the leaves of the grape cultivar, Italia.

5.2. Survey and documentation of natural enemies of *Dysmicoccus brevipes*

Among the total number of natural enemies collected from different locations of Ernakulam, Idukki and Thrissur district during January to May 2016, *Scymnus* sp. was the most abundant predator with 68.75 per cent in all the locations (Table 8). Avre *et al.* (2011) observed that the increase in the population of *Scymnus coccivora* was proportional to the incidence of *Phenococcus solenopsis* infesting on hibiscus plant commencing first fortnight of October (0.36 per 25 plants) and reaching the peak during second fortnight of November (1.12/25 plants).

Incidence of *Spalgis epius* accounted for about 17.61 per cent of the total insect natural enemies collected from all the locations. Thangamalar *et al.* (2010) observed large number of *S. epius* in the mulberry ecosystem infested by *Paracoccus marginatus* especially between June to October when other natural enemies were absent, while declined during October and November, when the presence of other natural enemies like *Cryptolaemus montrouzeiri* and *Scymnus* sp. were abundant. This finding supports the reason for low population of *S. epius* admist high population of *Scymnus* sp. Cham *et al.* (2013) also reported high number of *S. epius* in the papaya plantation infested with *Paracoccus marginatus* accounting for an average of 35 larvae between September 2010 to March 2011, whereas 80 per cent of *S. epius* was collected during January and February. The minimum occurrence of *S. epius* during the earlier months was due to the low incidence of mealybugs.

The drosophilid predator, *Cacoxenus perpsicax,* accounted for 7.39 per cent of the total insect natural enemies collected from few places *viz.*, Nadukkara,

Thodupuzha and Vellanikkara. Goolsby *et al.* (2002) also collected *C. perspicax* on the *Maconellicoccus hirsutus* from different location in Australia and found to be density dependent. Similarly, Sundararaj (2008) collected few numbers of *C. perspicax* from spherical mealybug, *Nipaecoccus viridis* infesting on sandalwood.

Chartocerus sp., was also recorded (6.25%) from Vazhakulam and Vellanikkara. Beltra *et al.* (2012), recorded sporadic occurrence of about 0.9 per cent of *Chartocerus* sp. of the total number of parasitoid collected.

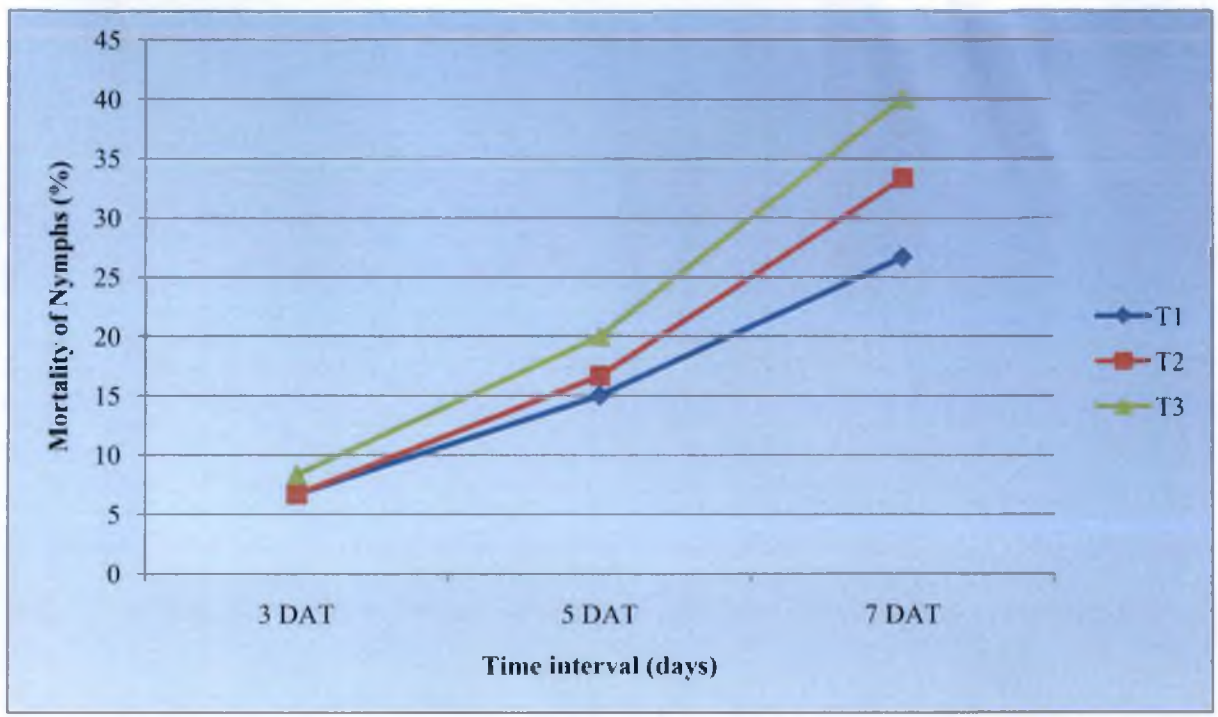
During the survey, two ant species *viz.*, *Camponotus mitis* and *Technomyrmex albipes* were found symbiotically associated with *D. brevipes*. Similar association by *Camponotus compressus* in arecanut plant infested with *D. brevipes* (Basavaraju *et al.* 2013) and *Paracoccus marginatus* with *C. compressus* and *Technomyrmex albipes* (Gowda *et al.*, 2014) were reported.

5.3 Evaluation of entomopathogenic fungi under laboratory conditions

Mortality of nymphs increased with the increase in period of exposure (Fig. 2 to Fig. 4). The highest mortality was recorded @ $1x10^9$ spores ml^{-1} concentration in all the entomopathogenic fungi, followed by the lower concentrations *viz.*, $1x10^8$, $1x10^7$ spores ml^{-1} which were on par with each other (Table 11). Saranya *et al.* (2010) also reported that the mortality of *Aphis craccivora* increased with the time. Seven days after treatment, *V. lecanii* @ 10^8 spores ml^{-1} everted 100 per cent mortality, followed by *B. bassiana* and *M. anisopliae* with 96.66 and 80.76 per cent mortality which was on accordance with the present finding.

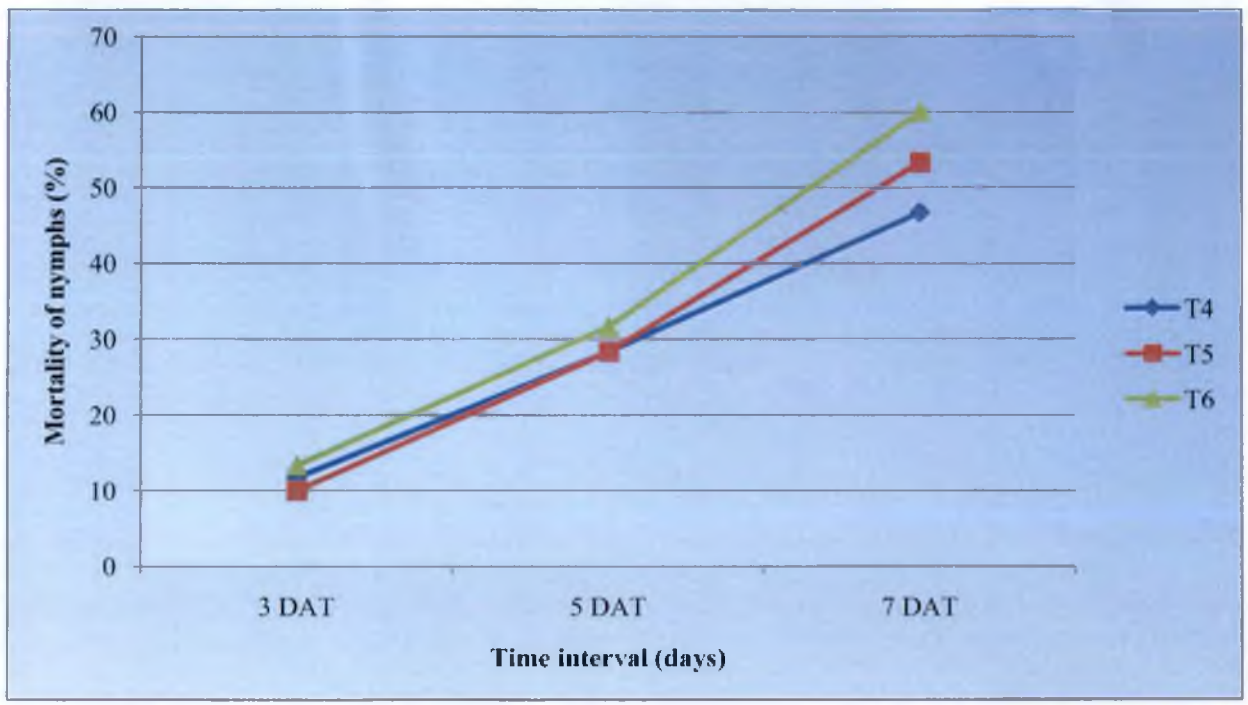
The highest mortality of mealybug was recorded (66.67%) in *L. lecanii* (10^9 spores ml^{-1}) followed by *B. bassiana* (10^9 spores ml^{-1}) with 60 per cent mortality of nymphs which were on par with each other. *M. anisopliae* had resulted in 40 per cent mortality. The present finding was in agreement with results of Banu *et al.* (2010) where, nymphs of *Paracoccus marginatus* were more susceptible to entomopathogenic fungi than adults, leading to the mortality of

Figure 2. Effect of concentrations of *Metarhizium anisopliae* on *Dysmicoccus brevipes* at different under laboratory conditions



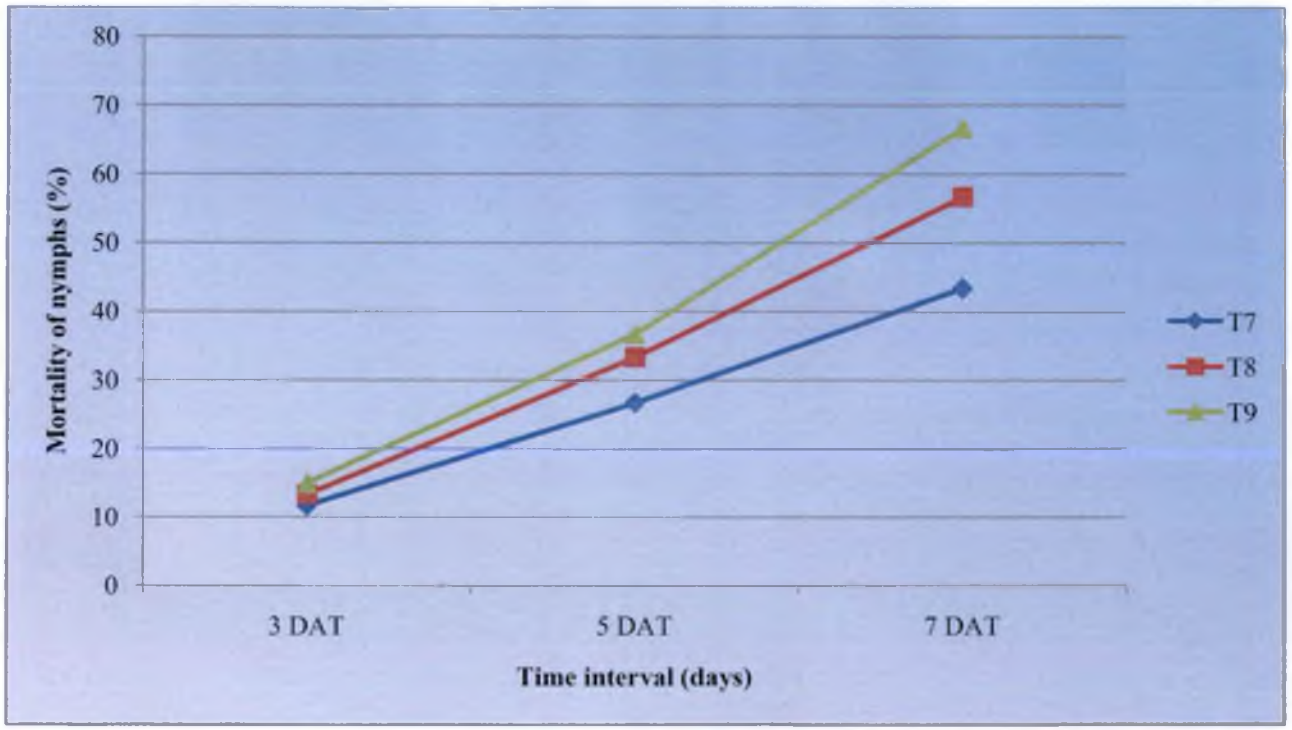
T1- *M. anisopliae* ($1x10^7$ spores ml^{-1}), T2- *M. anisopliae* ($1x10^8$ spores ml^{-1}), T3- *M. anisopliae* ($1x10^9$ spores ml^{-1})

Figure 3. Effect of different concentrations of *Beauveria bassiana* on *Dysmicoccus brevipes* under laboratory conditions



T4- *B. bassiana* ($1x10^7$ spores ml^{-1}), T5- *B. bassiana* ($1x10^8$ spores ml^{-1}), T6- *B. bassiana* ($1x10^9$ spores ml^{-1})

Figure 4. Effect of different concentrations of *Lecanicillium lecanii* on *Dysmicoccus brevipes* under laboratory conditions



T7- *L. lecanii* ($1x10^7$ spores ml^{-1}), T8- *L. lecanii* ($1x10^8$ spores ml^{-1}), T9- *L. lecanii* ($1x10^9$ spores ml^{-1})

51.11, 44.45 and 44.44 per cent with the spraying of *V. lecanii*, *B. bassiana* and *M. anisopliae*, respectively (2×10^8 cfu/gm) @ 5g/l. It might be due to the fact that wax covering on body of the nymphs was lesser than the adults which made the easy degradation of the cuticle by hydrolytic enzymes secreted by the fungus and hyphal penetration.

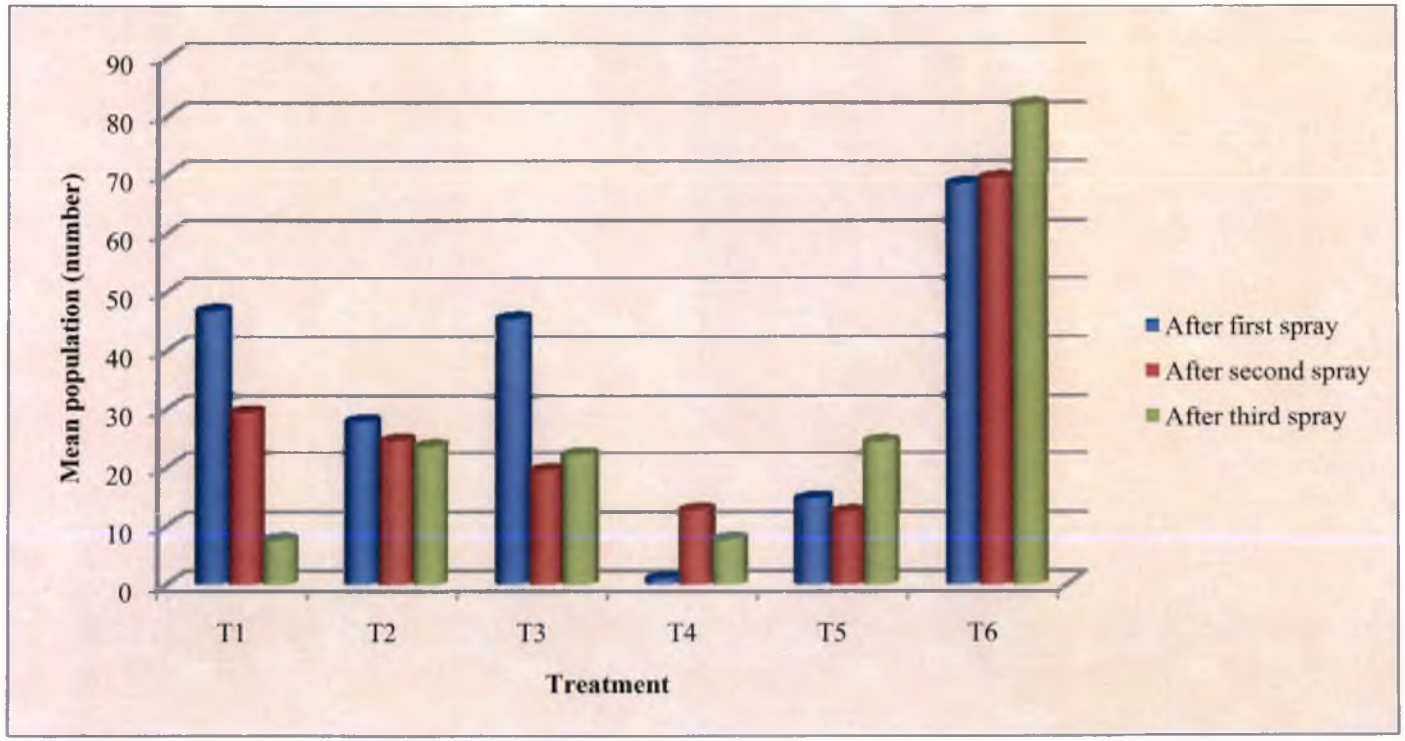
Makadia *et al.* (2009) also reported that, first and second instar nymphs of *Maconellicoccus hirsutus* were more susceptible to *V. lecanii* (2g/l) than the later instars. Ten days after entomopathogenic fungal treatment, the highest mortality of 65.10 and 58.99 per cent of the first and second instars nymphs of *M. hirsutus* was recorded, whereas 45.96 and 30.66 per cent mortality in third instar nymphs and adult mealybugs. The present study was found to be in conformity with the finding of Halder *et al.* (2013), where the per cent mortality of nymphs increased with the increase in time where six days after the spraying *V. lecanii* (@ 2×10^9 cfu/g) resulted in the mortality of 67.11 per cent followed by *B. bassiana* (1×10^8 cfu/g) and *M. anisopliae* (1×10^8 cfu/g) with 62.85 and 56.52 per cent mortality, respectively. Among the three tested fungal pathogens *L. lecanii* @ 1×10^8 spores ml^{-1} was proved to be efficient entomopathogen with increased efficiency as the period prolongs.

5.4 Evaluation of entomopathogenic fungi under pot culture experiment

Efficacy of the *Lecanicillium lecanii*, *Beauveria bassiana* and *Metarhizium anisopliae* @ $1x10^8$ spores ml^{-1} (three sprays) in reducing the population of mealybugs was evaluated with the three sprays of azadirachtin (1% @ 0.005%) and a standard check quinalphos (25 EC @ 0.05%) with a single spray. The observation on the per cent reduction of the mealybugs was taken at 10 days after each treatment.

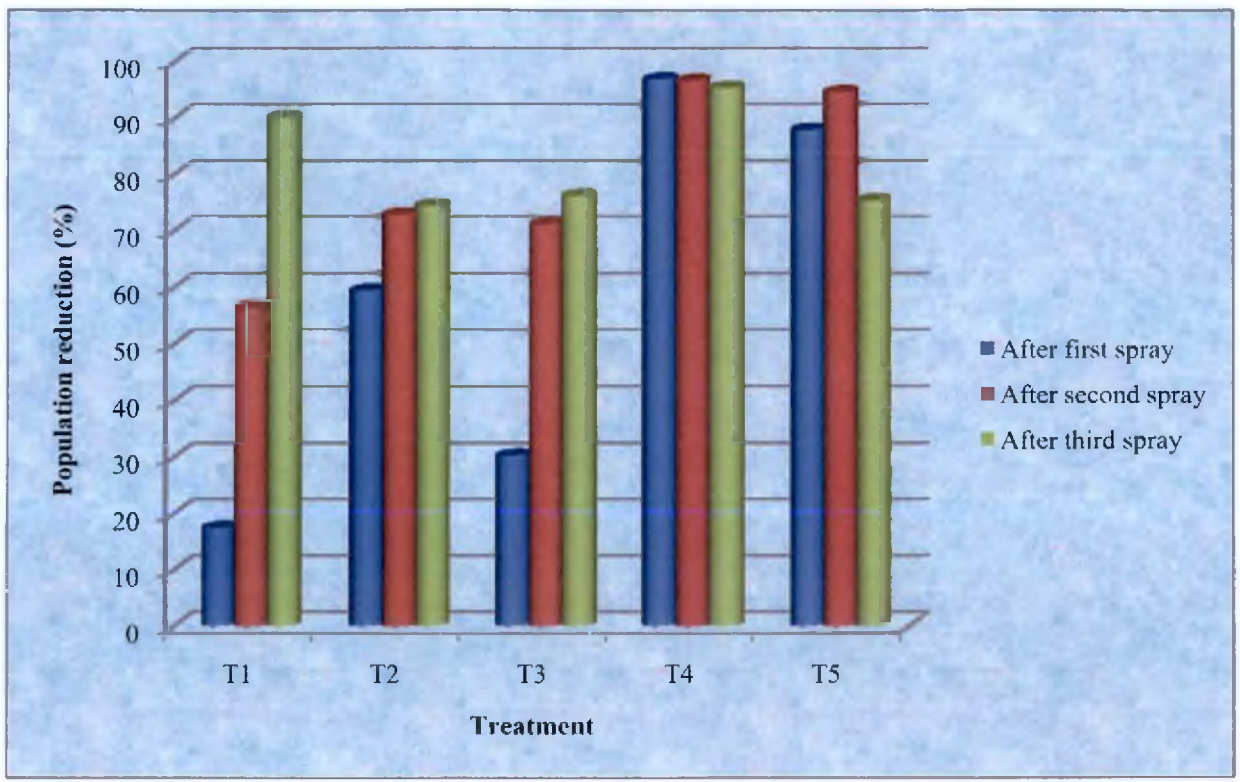
Single spray of quinalphos was the most effective in reducing the number of mealybugs upto 40^{th} day after the first spraying (Fig. 5 and 6). After the first spray, the highest reduction of 96.73 and 87.75 per cent mortality of mealybug was recorded with quinalphos and azadirachtin, respectively. It might be due to

Figure 5. Effectiveness of the entomopathogenic fungi, azadirachtin and quinalphos on *Dysmicoccus brevipes* in pot culture experiment



T1- *L. lecanii* @ $1x10^8$ spores ml^{-1}, T2- *M. anisopliae* @ $1x10^8$ spores ml^{-1} ,T3- *B. bassiana* @ $1x10^8$ spores ml^{-1} ,T4- Quinalphos 25EC @0.05 %, T5- Azadirachtin 1% @ 0.005%, T6-Control

Figure 6. Per cent reduction in population of *Dysmicoccus brevipes* over control by entomopathogenic fungi in pot culture experiment



T1- *L. lecanii* @ $1x10^8$ spores ml^{-1}, T2- *M. anisopliae* @ $1x10^8$ spores ml^{-1} ,T3- *B. bassiana* @ $1x10^8$ spores ml^{-1} ,T4- Quinalphos 25EC @0.05 %, T5- Azadirachtin 1% @ 0.005%

the fact that chemical insecticides and botanicals were the most effective in controlling the mealybugs, because of their immediate action on interrupting the physiology of the insects. Efficiency of the insecticide was proved earlier by Kumar *et al.* (2011) where the maximum mortality of 58.66 and 61.33 per cent when acephate (75% SP @ 5 ml/l) and chlorpyrifos (20% EC @ 8 ml/l) were sprayed, and Halder *et al.* (2013) who obtained a mortality of 70.29 per cent of the *Phenococcus solenopsis* on application of neem oil (5%) while entomopathogenic fungi recorded the least mortality (62.85, 56.52 and 67.11 per cent with *B. bassiana*, *M. anisopliae* and *V. lecanii*, respectively) over the control which was in conformity with the present finding.

Among the microbial pesticides, after the first application *M. anisopliae* recorded the maximum reduction of the mealybugs with 59.29 per cent mortality followed by *B. bassiana* (30.13%) which was on par with each other. *L. lecanii* recorded a mortality of 17.26 per cent. The present finding was in conformity with the report of Pandher *et al.* (2012), where seven days after the application of treatment *B. bassiana* @ 10 g/l recorded the maximum mortality (34%) of *P. solenopsis* followed by *M. anisolpliae* (28.88%) and *V. lecanii* (25.38%). The results of the present study could also be supported by finding of Amutha and Banu (2015) where they observed variation in the time and duration of various phases of mycosis of the *M. anisopliae*, *B. bassiana* and *V. lecanii* against the adults of the *P. marginatus*. *M. anisopliae* and *B. bassiana* were reported to possess rapid adhesion and penetration of the conidial spores on integument could be within 24 h and 48 to 96 h, respectively after the spraying of the fungus, whereas, in *V. lecanii,* hyphal penetration requires 48 h after the spraying lasting for 120 h. As the time required for the infection of *V. lecanii* was more, time required for causing the mortality of mealy bugs increased.

After the second spray, azadiractin gave the maximum mortality of 94.44 per cent of the mealy bugs. Among three EPF sprayed *M. anisopliae* recorded the maximum mortality of 72.72 per cent followed by *B. bassiana* (70.98%) which was on par with each other. The present result was in agreement with the findings

Surulivelu *et al.* (2012) who observed the maximum reduction of the *P. solenopsis* infesting on Bt cotton with 93.8 and 87.1 per cent mortality with synthetic insecticide acephate and chlorpyrifos, while moderate level with *B. bassiana*, *V. lecanii* and *M. anisopliae* resulting in 39.1, 30.9 and 28.2 per cent mortality, respectively.

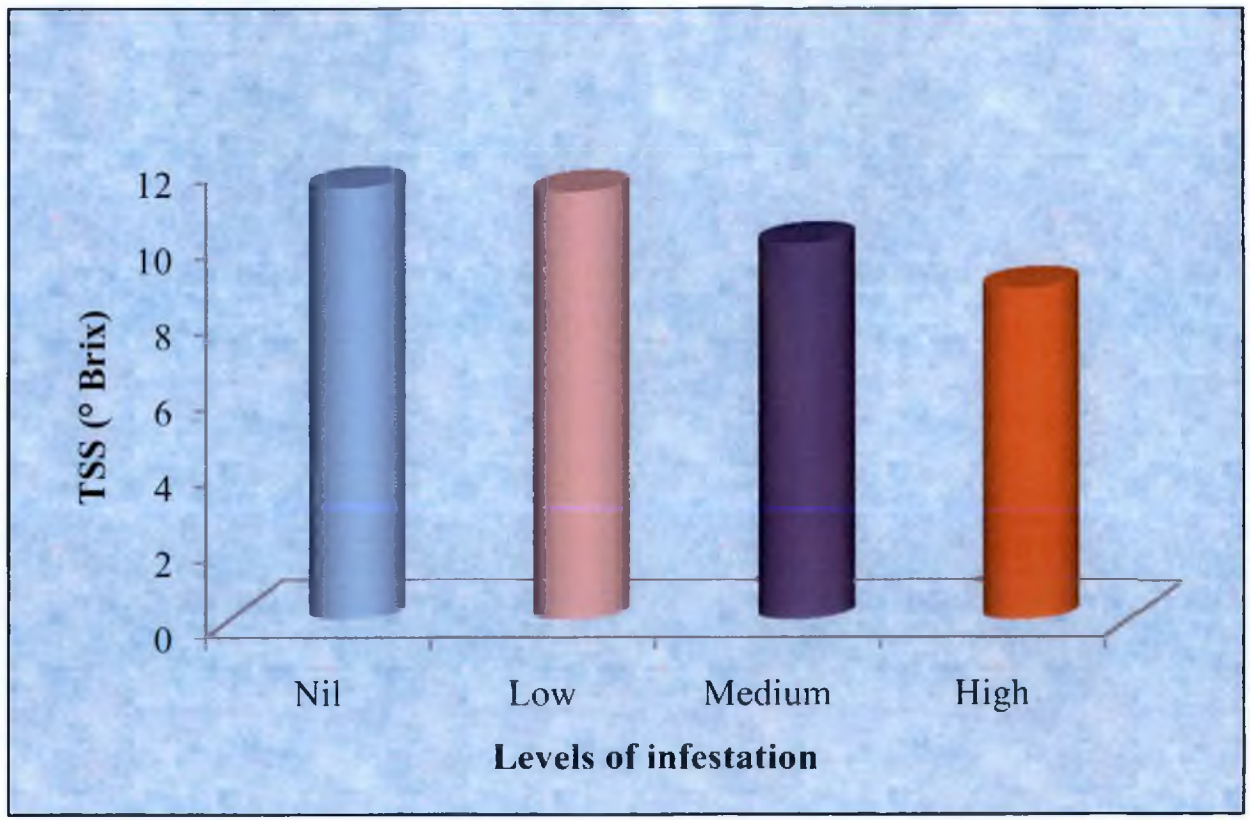
After the third spray, *L. lecanii* had the highest per cent mortality (90.04%) of mealybugs which was on par with the effect of quinalphos (95.2%). Effect of *L. lecanii* was followed by *B. bassiana* and *M. anisopliae*. Ghelani *et al.* (2014) also reported that *V. lecanii* @ 2.5 kg/ha gave the highest mortality of 48.2 per cent mortality of the *Aphis gossypii* infested on Bt cotton, while 44.6 and 37.7 per cent of mortality was obtained with the spraying of *B. bassiana* @ 2.5 kg/ha and neem oil (1%), respectively which was in line with the present findings. As observed in the laboratory experiments, *L. lecanii* was found to be the most effective as that of synthetic insecticides quinalphos after three sprays.

5.6 Impact of infestation on fruit quality

The TSS of the pineapple fruits with low level of mealybugs infestation and fruits without mealybug infestion did not differ significantly and was on par with each other. However, TSS in the pineapple fruits with medium and higher level of infestation differed significantly with the TSS of 9.93° and 8.74° Brix, respectively (Fig. 7). The present result was similar to the observation of Kumar *et al.* (2003) who reported that the TSS of the guava fruits got reduced with the level of infestation of tea mosquito bug, *Helopeltis antonii*. The TSS value of 12.58° Brix was noticed from 25 per cent infested fruit followed by 11.58, 10.27 and 9.1° Brix with 50, 75 and 100 per cent infested fruits, respectively.

Zubair *et al.* (2015) also reported the attack of sucking pest on the kinnow mandarin fruit (*Citrus reticulata blanco*) showed a negative association where the TSS of the fruits were reduced with increased in the number of sucking pest. It might be due to the attack of insects that caused the reduction of the transportable

Figure 7. Effect of *Dysmicoccus brevipes* infestation on Total Soluble Solids (TSS) in pineapple fruits



carbohydrates which was important in the development of the fruits and synthesis of juice in the fruits.

Summary

6. SUMMARY

The study entitled "Eco-friendly management of pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae)" was carried out in the Department of Agricultural Entomology, College of Horticulture, KAU Vellanikkara. The present study aimed at developing ecologically sound management practices to control the pineapple mealybug, *Dysmicoccus brevipes* a major threat to the pineapple cultivation throughout the world. The results obtained from the current study are summarized below.

Biology of *Dysmicoccus brevipes* was studied on pineapple leaf bits. In the present study males were absent and females produced through parthenogenesis. Morphometric observation and presence of moulting exuvia helped in distinguishing the different instars and duration between three instars. Average nymphal duration was about 40.2 days. The first instar nymphs were concealed beneath the female's body and were pinkish in colour, with the presence of six segmental antennae. The duration of the first instars lasted for 10.8 days. The first instar nymphs measured an average of 0.43 mm in length and 0.24 in width.

Second instar nymphs 0.67 mm in long and 0.34 mm in wide on an average were pink and flat with six segments in antennae. The second instars lasted for 13.7 days. The third instar nymphs were also flattened with pinkish body. Presence of seven antennal segments was another distinguishing character from the earlier instars. Third instars nymphs measured on an average of 1.05 mm in length and 0.785 mm in width and their duration lasted for 15.6 days.

Adult mealybugs were convex, pinkish and covered with thick layer of waxy coating. Antennae of adults were long with eight segments. Adults were 1.776 mm in long and 1.1 mm in wide. Adults lived for an average of 23.2 days. Pre-larviposition period of 8.7 days was observed. Individual females produced an average of 144.5 nymphs within a period of 4.5 days and post-larviposition period of 9.8 days was noticed. The total life cycle of adult female completed with an average of 63.4 days.

Number of antennal segments could serve as an important character to distinguish first, second, third instar and adults

Survey conducted at selected localities of Ernakulam, Idukki and Thrissur districts of Kerala from January to May, 2016 at monthly intervals revealed five species of insect natural enemies and a fungal pathogen from mealybug colonies *viz., Cacoxenus perspicax* (Drosophilidae), *Spalgis epius* (Lycaenidae), *Scymnus* spl and 2., a parasitoid *Chartocerus* sp. (Signiphoridae) and fungus *Apergillus* sp. *Scymnus* spp. was the predominant by *Spalgis epius*. Apart for the natural enemies, ant species *Camponotus mitis* (Smith) (Formicidae: Formicinae) and *Technomyrmex albipes* (Smith) (Formicidae: Dolichoderinae) were also found associated with *Dysmicoccus brevipes.*

Bioassay of the three entomopathogenic fungi *viz., Lecanicillium lecanii*, *Beauveria bassiana* and *Metarhizium anisopliae* under laboratory condition was evaluated at three different concentration ($1x10^7$, $1x10^8$ and $1x10^9$ spores ml^{-1}). At the highest concentration of $1x10^9$ spores ml^{-1}, the highest mortality of 66.67 per cent was recorded with *L. lecanii* , followed by *B. bassiana* (60 %) and *M. anisopliae* (40%) were on par with the succeeding lower concentrations. Hence the spore load of $1x10^8$ spores ml^{-1} could be effective to cause the desirable mortality.

Effectiveness of *L. lecanii, B. bassiana* and *M. anisopliae* ($1x10^8$ spores ml^{-1}) was evaluated in pot culture experiment in camparision with botanicals (azadirachtin 1% @ 0.005%) and a standard check (quinalphos 25EC @ 0.05%). After the first spray, quinalphos and azadirachtin treatment showed a drastic reduction of 96.73 and 87.75 per cent, respectively. *M. anisopliae* treatment was observed to be most effective resulting in greater reduction of mealybugs (59.29 %), followed by *B. bassiana* (30.13 per cent) and *L. lecanii* (17.26 per cent). Effects of all the entomopathogenic fungus in the reduction of mealybugs was found on par with each other.

Azadirachtin recorded the highest reduction of mealybug followed by *M. anisopliae* (72.72%) and *B. bassiana* (70.98) after the second spray. However, *L. lecanii* after the third spray resulted in maximum reduction of mealybugs (90.04 %) which was on par with quinalphos while, the effect of *B. bassiana* (76.04 %), azadirachtin (75.23%) and *M. anisopliae* (74.36%), were at par.

Variation in TSS of the affected pineapple fruits was assessed. Reduction of fruit quality in terms of TSS was observed to be directly related to infestation level as the TSS was 8.74° Brix in heavily infested compared to 9.93° Brix in moderately infested fruit, indicating the importance of mealybug infestation on quality of fruits.

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*Originals not seen

Appendix

APPENDIX- I

Weekly mean weather parameter during the period from April 2016 to June 2016

Weeks	Temperature		Relative humidity	
	Minimum (°C)	Maximum (°C)	Minimum (%)	Maximum (%)
1	23.02	32.74	49.71	72.85
2	22.50	32.56	60.43	78.14
3	22.68	33.21	66.28	81.71
4	24.23	34.34	54.43	74.14
5	22.84	32.86	56.43	69.28
6	24.41	31.24	60.14	72.71
7	23.34	32.41	61.43	71.28
8	23.38	32.87	58.28	75.71
9	22.84	32.11	58.43	77.14
10	22.58	32.21	59.28	79.42
11	22.42	31.82	63.00	78.43
12	24.14	30.98	60.71	78.00
13	23.30	30.75	64.57	79.28

ECO-FRIENDLY MANAGEMENT OF PINEAPPLE MEALYBUG *Dysmicoccus brevipes* (COCKERELL) (HEMIPTERA: PSEUDOCOCCIDAE)

By
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(2014-11-214)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement

for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

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VELLANIKKARA, THRISSUR – 680 656

KERALA, INDIA

2016

ABSTRACT

Pineapple mealybug (*Dysmicoccus brevipes*) causes severe damage to pineapple crop in Kerala. Apart from the direct damage and it also transmits Pineapple Mealybug Wilt Disease (PMWD). This is a polyphagous pest and its host includes banana, coffee, citrus, palm, sugarcane *etc.* Use of broad spectrum synthetic insecticides to manage the mealybug has been restricted owing to the residual problem in the fruit and other environmental concerns. Hence, the present study, "Eco-friendly management of pineapple mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae)" was carried out at Department of Agricultural Entomology, College of Horticulture, Vellanikkara, during September 2014 to June 2016, with the objectives to document the natural fauna of *Dysmicoccus brevipes* and to formulate eco-friendly measures for managing the pest polulation

Laboratory rearing of *D. brevipes* was done on pumpkin fruits. The mealybugs reproduced parthenogenetically. Males were absent in the population. Life cycle of females consisted of three nymphal instars and adult stage. The mean duration of first and second nymphal instars were 10.8 and 13.7 days, respectively. The average third nymphal instar period was 15.6 days. Adult females lived for an average of 23.2 days with pre-larviposition, larviposition and post-larviposition period of 8.7, 4.5 and 9.8 days, respectively. An adult female deposited an average of 144.5 crawlers. The total life cycle completed within 63.4 days. Number of antennal segments varied among each instars. The first and second instar nymphs had six antennal segments each while third instar nymphs and adult possessed seven and eight antennal segments respectively.

To document the natural enemies of *D. brevipes*, purposive survey was done in selected locations of Ernakulam, Idukki and Thrissur districts. The natural enemies recorded included four predators [*Spalgis epius* (Westwood), *Cacoxenus perspicax* (Knab) and two unidentified species of *Scymnus*] and one parasitoid (*Chartocerus* sp.).

Three species of Entomopathogenic fungi (EPF) *viz., Metarhizium anisopliae, Beauveria bassiana* and *Lecanicillium lecanii* were evaluated at three different concentrations ($1x10^7$, $1x10^8$ and $1x10^9$ spores ml^{-1}) under laboratory conditions against *D. brevipes*. Highest spore concentration of all the entomopathogenic fungi resulted in higher mortality of mealybugs. *L. lecanii* @ $1x10^9$ spores ml^{-1} concentration had resulted in 66.67 per cent mortality. Similarly, in case *B. bassiana* and *M. anisopliae* @ $1x10^9$ spores ml^{-1} concentration mortality of 60 and 40 per cent, respectively was observed.

In pot culture studies, the best performing concentration of EPF from the laboratory assay was evaluated along with a botanical insecticide and a standard check (quinalphos 25EC @ 0.05%). Ten days after the first treatment application, highest reduction was observed in quinalphos (96.73%) followed azadirachtin (87.75%). Among the three EPF tested, *M. anisopliae* caused the maximum reduction of (59.29%) of mealybug. Similarly, second spray of *M. anisopliae* and *B. bassiana* recorded maximum reduction of 72.72 and 70.98 per cent, which were statistically on par with each other. After the third spray, *L. lecanii* resulted in highest reduction (90.04%) of mealybugs which was on par with the reduction obtained by quinalphos (95.20%) application.

Infestation of *Dysmicoccus brevipes* induced changes in pineapple fruit quality and it was estimated by quantifying the Total Soluble Solids (TSS), content of the fruit at varied level of infestation. Fruits with heavy infestation of mealybugs showed less TSS content (8.74° brix) compared to the TSS level in medium and low infestation (9.93° brix and 11.31° brix, respectively).