POPULATION DYNAMICS, BIOLOGY AND MANAGEMENT OF MEALYBUG, *Phenacoccus solenopsis* Tinsley (HEMIPTERA: PSEUDOCOCCIDAE) ON OKRA

By

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(2015 – 11 - 113)

THESIS

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DECLARATION

I, Anusree Padmanabhan P. S. (2015-11-113), hereby declare that this thesis entitled **"Population dynamics, biology and management of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on okra"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me any degree, diploma, fellowship or other similar title of any other University or Society.

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Introduction

1. INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench) is a tropical vegetable crop belonging to the family Malvaceae. Okra is considered to be West African, Ethiopian and South Asian in origin. Okra pods are harvested immature, cooked, pickled, included in salads or eaten raw. The seeds can be roasted and ground to prepare a caffeine-free substitute for coffee. Okra is an excellent fiber source to help maintain a healthy digestive system. It provides 43 per cent of the recommended dietary allowance (RDA) of manganese and 36 per cent of vitamin C. It also contains potassium (299 mg/100g), calcium (82mg/100gm), magnesium (57mg/100g) and carbohydrates (7.45mg/100g). Biodiesel is derived from okra seeds (up to 12% w/w) (Anwar *et al.*, 2010). Bast fibers are also extracted from the stem of okra which has industrial values.

Okra is one among the most important vegetable crop grown in India, Nigeria, Pakistan, Cameroon, Iraq and Ghana having worldwide production of 7896.3 thousand tons covering an area of 1148.0 thousand hectares. In India, it covers an area of 507.45 thousand hectares with an annual production of 5853.02 thousand tons. West Bengal has the largest area under okra cultivation (75.45 thousand ha) followed by Gujarat (65.99 thousand ha) and Odisha (64.63 thousand ha). West Bengal ranks first in okra production with an annual production of (882.39 thousand tons) followed by Bihar (762.90 thousand tons) and Gujarat (75). Arka Anamika, Arka Abhay, Pusa Makhmali, Pusa Sawani, Parbhani Kranti and Punjab no. 13 are the major varieties of okra cultivated in India.

In Kerala, okra is cultivated in 2030 hectares with a production of 13.75 thousand tons of pods. The major okra growing districts of Kerala are Palakkad (415 ha), Kollam (165 ha), Malappuram (76 ha) and Thiruvananthapuram (59 ha) followed by Ernakulam (46 ha) and Thrissur (40 ha) (AEZ, 2001).

The production and productivity of okra in India are lower compared to other countries due to yield losses caused by insect pests, diseases and nematodes. Major insect pests attacking okra are cotton mealybug, (*Phenacoccus solenopsis* Tinsley), shoot and fruit borer (*Earias vittella* Fabricius), fruit borer (*Helicoverpa armigera* Hubner), leaf hopper (*Amrasca biguttula biguttula* Ishida), whitefly (*Bemisia tabaci* (Gennadius)) and aphid (*Aphis gossypii* Glover). Among these pests, the cotton mealybug has been reported as a serious pest in Kerala. The infestation starts at early stages of the crop and often leads to death of the plant. Even if the plant survives the infestation drastically reduces the market value of okra pods.

Mealybug, *P. solenopsis* (Hemiptera: Pseudococcidae) a native of USA that coevolved with various host plants, has become a highly invasive and polyphagous. It reportedly damages more than 200 plant species in economically important families such as Malvaceae, Solanaceae, Cucurbitaceae and Fabaceae (Fand and Suroshe 2015). The pest received worldwide attention as an invasive pest of quarantine importance. In India, it is a major pest of cotton, while in Kerala it is reported to cause serious damage to okra (*A. esculentus*) and China rose (*Hibiscus rosa-sinensis* L.).

The mealybug sucks large amount of sap from leaves and stems of host plants, depriving plants of essential nutrients. The plants become retarded, leaves crinkled and show yellowing symptoms. Infested flowers drop and produce no fruits. The quality of okra pods will be reduced due to excessive sucking of cell sap. Mealybug produces copious amount of honeydew which creates sooty mould growth which in turn reduce the photosynthetic efficiency of the plant.

P. solenopsis is known to be highly cryptic in nature, which makes species identification difficult. A number of *P. solenopsis* clades have been reported from various parts of the world. Management of mealybug is difficult as it has a wide host range, high reproduction potential and waxy coating on the body.

In this context, it is necessary to understand the mealybug population status infesting okra in Kerala. The life history of mealybug should be thoroughly studied to develop effective management strategies. The information on predator and parasitoids of mealybug should be collected and thoroughly studied. There is a necessity to confirm the species level identity of mealybug collected from the host plants due to their cryptic nature. Study on endosymbiotic relations of mealybug with other microorganisms may help to reveal their polyphagous nature, enzymes helping in biomass deconstruction *etc*. Evaluation of new and safer insecticide molecules along with conventional pesticides and biocontrol agents should be done to develop economically feasible and more viable integrated pest management practices. Hence the present study is proposed with the following objective,

- To study the population dynamics, biology and management of *P. solenopsis* Tinsley and characterization of its endosymbionts.

Review of literature

2. Review of Literature

Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has been regarded as a major pest infesting okra (*Abelmoschus esculentus* L. Moench). This is highly invasive and polyphagous pest infesting vegetable crops, fruit crops, ornamentals and numerous weed plants. The available literature related to population dynamics, host range, biology, damage symptoms, management and molecular characterization of *P. solenopsis* is reviewed here under.

2.1 Population dynamics, distribution and host range of cotton mealy bug, *Phenacoccus solenopsis*

Tinsley (1898) first described the existence of mealybug, *P. solenopsis* from New Mexico and the specimens were collected from infested roots and stems of weeds *viz.,* creeping spiderling (*Boerhavia spicata* Choisy), California caltrop (*Kallstroemia californica* (S. Wats.) Vail) and also from the nest of ants, *Solenopsis geminata* (Fabricius).

Fuchs *et al.* (1991) reported first known occurrence of the cotton mealybug *P. solenopsis* infesting cotton in USA. It was also found to be associated with 29 other plant species of 13 different families. Other plants infested by the cotton mealybug were *Solanum muricatum* Ait. (Larrain, 2002) and *Solanum lycopersicum* L. (Culik and Gullan, 2005). Granara de Willink (2003) recorded the presence of mealybug from Argentina on false ragweed, *Ambrosia tenuifolia* Spreng.

Tanwar *et al.* (2007) recorded heavy infestation of mealybug on cotton crop in Maharashtra, Punjab, Rajasthan and Gujarat and also sudden appearance of the pest in Multan, Sanghar, Mirpurkhas and Tando Allahyar of Pakistan had almost destroyed the crop within very few days.

Hodgson *et al.* (2008) recorded serious outbreak of *P. solenopsis* on both Bt and non-Bt cotton growing areas of Pakistan (Sindh and Punjab) namely, Bahawal

Pur, Dera Ghazi Khan, Faisalabad, Layyah, Lodhran, Multan, Muzaffar Garh, Nawab Shah, Rajan Pur, Toba Tek Sing, Khanewal, Rahim Yar Khan, Bahawal Nagar, Vehari, Lodhran, Sanghar, Tandojam and Mir Par Khas. Suresh *et al.* (2007) found that the population density of *P. solenopsis* varied from 0 to 20 per 5 cm twig, with a peak population between April and May. Population of mealybug on *Hibiscus rosa-sinensis* L. was moderate and the incidence extended to August, While on *Parthenium hysterophorous* L. the mealybug population present throughout the year.

Durgaprasad *et al.* (2008) made several surveys in Andhra Pradesh and found that 50 to 75 per cent fields of the state were infested with mealybug with damage ranging from 1 to 5 per cent. Dominant species identified were *P. solenopsis* and *Maconellicoccus hirsutus* Green. Akintola and Ande (2008) conducted surveys in Nigeria and reported the occurrence of cotton mealybug, *P. solenopsis* for the first time on China rose, *Hibiscus rosa-sinenesis* L. where the adult females were aggregated on the stem of the plant.

Thomas and Ramamurthy (2008) collected mealybugs from different parts of India and reported the abundance of mealybug, *P. solenopsis.* They also revealed that there were confusions in identification of mealybug due to misidentifications, synonymy and interpretation of taxonomical characters, mainly the multilocular disc pores and circulus in the abdominal segments. Jhala *et al.* (2009a) reported the occurrence of *P. solenospsis* infesting cotton for the first time in Gujarat, where they recorded that, 25 to 30 per cent cotton fields of Gujarat were infested with the meaybug and 20 to 90 per cent of plants were adversely affected. The mealybug, *P. solenopsis* was introduced to India from USA via Pakistan and covered several states of India *viz.,* Punjab, Haryana, Gujarat, Maharashtra, Rajasthan and Madhya Pradesh (Jhala *et al.,* 2009b). Wu and Zhang (2009) reported the presence of mealybug *P. solenopsis* on *H. rosa- sinensis* for the first time from China.

Silva (2012) first time reported the occurrence of the mealybug *P. solenopsis* on cotton from the states, Bahia and Pariba of Brazil. Singh and Gandhi (2012) stated that, *P. solenopsis* as a serious threat to agricultural crops, considered to be infesting almost all agricultural crops and weed host due to their polyphagous feeding habit, during their surveys of Vadodara district Of Gujarat. Singh and Kumar (2013) conducted population studies of *P. solenopsis* in agricultural fields of Vadodara, Gujarat where they found that infestation started in the month of August and attained the maximum population in the month of October on cotton as well as okra. Whereas, population dynamic study conducted on tomato (*L. esculentum*) and potato (*Solanum tuberosum* L.) showed the peak level of mealybug population during the month of February.

Maruthadurai and Singh (2015) reported the presence of cotton mealybug, *P. solenopsis* on cashew from Goa for the first time. Based on reports, the peak infestation of the mealybug was recorded in the month of April to May, while mealybug population gradually reduced from July to August. Vilela *et al.* (2015) observed the presence of *P. solenopsis* for the first time on evergreen ornamental plants such as lantana (*Lantana camara* L.), blood leaf (*Iresine herbstii* Hook. ex Lindi) and pilea (*Pilea serpyllacea* (Kunth) Wedd.). Later the mealybugs were transferred to annual plants such as common purslane *(Portulaca oleracea* L.) and Moss-rose purslane, (*Portulaca grandiflora* Hook).

Ibrahim *et al.* (2015) recorded the presence of *P. solenopsis* on tomato plants for the first time in Egypt. The infestation appeared on the stem, leaves, terminal bud, flowers and they found to exhibit clear symptoms of deformation and distortion of terminal growth, foliar yellowing, leaf crinkling and puckering. Halder *et al.* (2015) documented *P. solenopsis* as a dominant mealybug species in vegetable ecosystems infesting several crops *viz.,* okra, tomato, capsicum, brinjal and pointed gourd and *P. hysterophorous*.

El-Zahi *et al.* (2016) reported the presence of *P. solenopsis* on cotton in Egypt for the first time. They found adult female mealybugs and nymphs on cotton leaves, fruiting buds, blooms and green bolls. Infested bolls were completely damaged due to heavy infestation. Beshr *et al.* (2016) conducted surveys on Alexandria and Behaira governorates of Egypt, which revelaed a total of 22 host plants from 18 families. Six vegetable crops found to be heavily infested with mealybug were, *Abelmoschus esculentus* L., *Corchorus olitorius* L., *Lycopersicum esculentum* L., *Solanum melongena* L. and *Portulaca oleracea* L. Infested okra plants showed a decrease in quality of yield produce, which reduced the market price.

2.1.1 Host range of *Phenacoccus solenopsis*

Deshmukh *et al.* (2009) conducted surveys for documenting the host range of cotton mealybug, *P. solenopsis* under the rainfed cotton cultivation areas which revealed about 91 host plants of mealybug distributed under 24 families [where *P. solenopsis* was found on 30 host plants during the cotton growing season and 61 other host plants during off season]. Plant species exclusively from three families *viz.*, Malvaceae, Compositae and Leguminosae found to constitute 50 per cent of host plants of *P. solenopsis*. Alternate host recorded during off season itself showed the ability of strong carry over between cotton seasons. Arif *et al.* (2009) documented 154 host plants from 53 families which include 20 field and horticultural crops, 45 ornamentals, 64 weeds and 24 bushes and trees. Plant species from Malvaceae, Solanaceae, Amaranthaceae, Ficoidae, Convolvulaceae, Euphorbiaceae, Verbanaceae and Zygophyllaceae were found the most preferred host for cotton mealybug.

Abbas *et al.* (2010) documented 55 host plants from 18 families infested by cotton mealybug *P. solenopsis*. Among them, tobacco, China rose, cotton and okra were found infested heavily by the mealybug.

Suroshe (2011) recorded peak population of mealybug on okra during October which declined from the onset of winter and showed a positive correlation with temperature. Sahito *et al.* (2011) reported the cotton mealybug infestation on datura, tomato, okra, sunflower, lantana, maize, winter cherry *etc.* And mealybugs were also reported from weeds like *Trianthema portulacastrum* L. and *Euphorbia prostrata* Aiton.

Arif *et al.* (2012) found that the peak populations of mealybugs were observed during March and April on vegetables, mainly okra and brinjal. Mealybug population was at peak level on cotton during October and November. Trend of mealybug infestation was almost the same on weeds throughout the year.

Ibrahim *et al.* (2015) reported the cotton mealybug as a polyphagous pest. They documented more than 200 plant species in 60 families as host plants of the mealybug. The cotton mealybug showed special preference for host plants belonging to Asteraceae, Euphorbiaceae, Fabaceae, Malvaceae and Solanaceae. Zhou *et al.* (2015) showed that the plant *Eupatorium adenophorum* L. facilitates the colony growth of *P. solenopsis*.

Satpathy *et al.* (2016) first time reported the presence of *P. solenopsis* on cultivated jute, *Corchorus olitorius* L. (Malvaceae) from many parts of Southern West Bengal in India. Zhou *et al.* (2015) evaluated resistance of ten cotton varieties against *P. solenopsis*. The study revealed that CCR159 had better resistance to *P. solenopsis*, while Bt cotton RCH-134 had no resistance to the mealybug.

2.2 Biology of *Phenacoccus solenopsis*

Hodgson *et al.* (2008) reported that *P. solenopsis* could overwinter successfully on a range of weeds and other plants. During the summer season, mealybug would be covered with elastic, white, sticky wooly wax mostly formed of the ovisacs of adult females. The crawlers would get easily dispersed to other parts of

the same plant or get carried by the wind or by other means like farm machinery, workers to other areas.

Parthenium stem cuttings were reported to be ideal for studying the biology of *P. solenopsis* as the plant was available throughout the year (Suroshe, 2011). Hameed *et al.* (2012) studied the effect of temperature and humidity on life history of mealybug, *P. solenopsis* which revealed the ability of the mealybug to develop and reproduce successfully at temperature 20 to 35±1°C and relative humidity (RH) 70 to 40±5%. Adult male longevity was less affected in accordance with the increase in temperature and decrease in humidity, while mealybug female showed strong variations in longevity period with the temperature and humidity fluctuations. According to Prasad *et al.* (2012) *P. solenopsis* exhibit obligate ovo - viviparous reproduction and the development of female and male nymphal instars linearly decreased with increase in temperature from 18 to 32°C.

Huang *et al.* (2012) used electrical penetration graph technique and an artificial membrane system to check whether *P. solenopsis* could imbibe free water or other liquid in its natural environment. The results showed that *P. solenopsis* continuously imbibe of water- honey solution for several hours and they concluded that there was no significant effect of imbibing water in increasing body size, yet it helped in extending their life span.

Sanghi *et al.* (2015) compared the longevity, ovarian development and number of eggs laid in mated and unmated individuals of *P. solenopsis* and the study showed that mated individuals laid more number of eggs, higher duration of gestation and duration of reproduction. Unmated individuals produced very few eggs. Zhou *et al.* (2015) measured biological parameters of *P. solenopsis* on *Eupatorium adenophorum* L. and two other cultivated plants (*Gossypium hirsutum* L. and *Hibiscus rosa-sinensis* L.), which showed that the populations on *Eupatorium*

adenophorum L. had a shorter developmental duration, greater fecundity, higher survival and larger populations.

El - Zahi *et al.* (2016) showed that the developmental durations of mealybug, *P. solenopsis* decreased with increase in temperature from 18°C to 30°C, while there were no significant change from 30°C to 34°C. Mealybug populations reared on potato and and China rose showed lower developmental durations compared to those reared on tomato and tobacco. Yuan *et al.* (2016) evaluated the fitness of mealybug *P. solenopsis* feeding upon healthy and diseased *H. rosa- sinensis*, infected with cotton leaf curl Multan virus (CLCuMV). They found that 1st and 2nd instar nymphs of mealybug preferred to feed on healthy plants compared to 3rd instar nymphs and female adult mealybugs, which had no preference between healthy and virus infected host plants. The fecundity of female adult mealybugs fed on virus infected plants got reduced by 47 per cent.

2.2.1 Incubation period

Kamariya (2009) found that the mealybug laid eggs in an ovisac of thin white cushiony pouch. The average incubation period was 6.62 ± 1.72 days. Ghulam *et al.* (2009) showed that the incubation period of *P. solenopsis* was 30 to 40 minutes at 25 ± 2 °C and a relative humidity of 70 ± 5 %. While Dhawan and Saini (2009) observed the incubation period of *P. solenopsis* on cotton was 1 to 2 days with a mean of 1.4 ± 0.5 days. According to Joshi *et al.* (2010) the incubation period of female mealybug was 4 to 6 hours. Kamariya and Patel (2011) reported that the average incubation period of *P. solenopsis* was 6.62 ± 1.71 days on cotton, while Kedar *et al.* (2011) reported that the incubation period of *P. solenopsis* was 2.25 to 4.83 minutes with an average of 3.21 ± 0.93 minutes on potato sprouts. Fand *et al.* (2010) reported that the female mealybug produced about 351± 44.73 young ones in her ovipositional period of 11.75 ± 0.96 days.

2.2.2 Nymphal period

Akintola and Ande (2008) recorded the developmental period of cotton mealybug *P. solenopsis*, which showed that there were three nymphal stages for female mealybug which lasted for six, eight and ten days and the total number of days from egg to adult longevity was about 37 days. The total nymphal period of mealybug, *P. solenopsis* was minimum 12 days and maximum 21 days and total duration of life cycle of *P. solenopsis* was minimum 25 days and maximum 36 days (Kamariya, 2009).

Vennila *et al.* (2010) recorded the developmental period of mealybug which was higher for male mealybug crawlers at 18.9 ± .0.9 days compared to female crawlers at 13.2 ± 1.8 days. Survival of second instar was lower (45 %) as compared with the first and third instars (75 %). Parthenogenesis with ovoviviparity was dominant (96 %) over oviparous (3.5 %) mode of reproduction. The reproductive period lasted from 30.2 ± 8.2 days. Kedar *et al.* (2010) recorded nymphal period of *P. solenopsis* as, 4.80 ± 0.83 days for 1st instar, 4.72 ± 0.68 days for 2nd instar and 5.20 ± 0.71 days for third instar at Hisar, Haryana. Nikam *et al.* (2010) recorded 6.70 ± 0.47 days of 1st instar, 4.70 ± 0.77 days of 2nd instar and 4.80 ± 0.65 days of 3rd instar of *P. solenopsis* at 28 - 30°C temperature and 75 to 80 per cent RH. Singh and Kumar (2013) showed that female mealybug had three nymphal stages and the adult female was wingless, while the male was winged and had two nymphal instars with an additional pupal instar.

2.2.3 Adult longevity

Aheer *et al.* (2009) reported that cotton mealybug, *P. solenopsis* had three nymphal instars in females and two nymphal instar with an additional pupal instar in male. Akintola and Ande (2008) recorded the longevity of mealybug, *P. solenopsis* on *H. rosa-sinensis* as 37 days. They also found that the reproduction in mealybug was bisexual and ovo-viviparous and adult male lived for 2 to 3 days while female mealybug lived for 45 to 85 days. While Ghulam *et al.* (2009) reported that, longevity of male mealybug on *H. rosa- sinensis* was 2 to 3 days and female mealybug was 45 to 85 days. Vennila *et al.* (2010) reported that Adult female lived for 42.4 ± 5.7 days whereas male lived only 1.5 ± 0.1 days. Arve (2010) reported that the average longevity of male and female was 2.60 ± 0.59 and 28.8 ± 2.60 days respectively. Joshi *et al.* (2010) concluded that the male mealybug lasted for 3 to 5 days while female mealybug lived for 30 to 48 days. Jat (2011) observed that the duration of male cocoon period of *P. solenopsis* was ranged from 4 to 6 days with an average of 5.00 ± 0.69 days, while Ghulam *et al.* (2009) reported that the male cocoon period of *P. solenopis* was ranged from 6.5 to 9 days. Dhawan and Saini (2009) recorded that the male cocoon period of *P. solenopsis* ranged from 6 to 8 days. Singh and Kumar (2013) reported that the *P. solenopsis* male was short lived with an adult life of only 1.96 ± 0.84 whereas female lived for 39.88 ± 3.12 days.

2.2.4 Pre-oviposition, oviposition and post-oviposition period

Dhawan and Saini (2009) reported the pre-oviposition, oviposition and post-oviposition period of mealybug, *P. solenopsis* on cotton as, 4.4 ± 0.4, 8.2 ± 0.8 and 2.6 ± 0.6 days respectively. Kedar *et al.* (2010) reported that the pre-oviposition, oviposition and post-oviposition period of the mealybug was observed to be 5.96 ± 0.73, 10.08 ± 1.12 and 3.00 ± 0.76 days respectively. Vennila *et al.* (2010) recorded mean pre-oviposition, oviposition and post-oviposition period of mealybug, *P. solenopsis* was 5.7 ± 1.7, 17.2 ± 4.3 and 2.4 ± 0.6 days respectively. Laboratory

studies of *P. solenopsis* from Junagadh revealed that the pre-oviposition, oviposition and post-oviposition periods of *P. solenopsis* were 4.32 ± 0.80, 8.00 ± 0.82 and 2.72 ± 0.79 respectively (Kamariya and Patel, 2011). Jat (2011) found that the pre - oviposition, oviposition and post-oviposition period of mealybug, *P. solenopsis* ranged from 4 to 8, 15 to 19 and 4 to 10 days with an average of 4.88 ± 1.02, 16.55 ± 1.09 and 7.90 ± 1.21 days, respectively.

2.2.5 Fecundity of mealybug

Radadia *et al.* (2008) observed that adult female of *P. solenopsis* laid on an average of 150-600 eggs when it was reared on cotton, while Dhawan and Saini (2009) recorded a fecundity of 270 to 340 with a mean fecundity of 308.6 ± 26.25 eggs on cotton in Punjab. Abbas *et al.* (2009) reported that, the mean fecundity of mealybug *P. solenopsis* was 122.5, 120.7 and 111.9 when reared upon cotton, China rose and okra respectively. According to Joshi *et al.* (2010) the fecundity of cotton mealybug was observed as 310 to 625 eggs per female with an average of 470 eggs. Fand *et al.* (2010) reported that the mortality rate of first instar crawlers was higher compared to other nymphal instars. Arve (2010) recorded the fecundity of mealybug, *P. solenopsis* on hibiscus leaves to be 256.16 ± 133.64 eggs. While Nikam *et al.* (2010) observed that the fecundity of *P. solenopsis* was 400 to 700 eggs with an average of 572 ± 102 eggs per female. Vennila *et al.* (2010) showed varied patterns of fecundity of mealybug, *P. solenopsis* ranging from 158 – 812 with an average of 344 ± 82. Kamariya and Patel (2011) observed that the fecundity of mealybug, *P. solenopsis* was 283 to 603 eggs with an average of 427.68 ± 86.69 eggs at 23.6 °C and 59.2 per cent relative humidity.

2.2.6 Total life cycle

The total life span of male and female of mealybug, *P. solenopsis* was observed to be 19.5 to 30 and 61.5 to 106 days respectively on China rose (Ghulam *et al.*, 2009). Dhawan and Saini (2009) reported that the life cycle of adult female and

male mealybug lasted 32.4 ± 4.4 and 20.5 ± 2.9 respectively on China rose. Joshi *et al.* (2010) reported that the life cycle duration of female mealybug was 27.25 ± 0.5 days at 27 ± 2 °C and 65 ± 5 % relative humidity. Kedar *et al.* (2010) recorded the total period of life cycle of male and female mealybugs of *P. solenopsis* on cotton in Haryana, where it was observed to be 19.25 ± 1.55 days and 33.79 ± 1.67 days respectively. Nikam *et al.* (2010) reported that the total life cycle of male and female mealybug was on an average of 26.73 ± 2.20 and 58.00 ± 3.72 days respectively in Gujarat. Jat (2011) reported that *P. solenopsis* completed its life span in 43 to 54 days with an average of 47 ± 2.50 days on tobacco.

2.3 Nature of damage by *Phenacoccus solenopsis*

Hodgson *et al.* (2008) reported that *P. solenopsis* is mainly found on the young growth including twigs, leaves, flower buds and petioles but can also occur even on the stem at heavy infestations. Infested plants appeared to be stunted, and dehydrated. In addition, the plants become covered with dense mat of sooty mould that grew on exuded honeydew which attracted ants that would probably defend the mealybugs from attack of parasitoids and predators.

Bhosle *et al.* (2009) conducted a study on incidence of cotton mealybug *P. solenopsis* in rainfed cotton cultivating areas of Marathwada. Severe infestation of mealybug was observed in Parbhani reigion with 40.95 per cent leaf infestation and 35.77 per cent green boll damage. The cotton mealybug was found mainly colonizing on young growth including stems, leaves, twigs and fruiting bodies. Heavily infested plants became stunted with no further growth. Sooty mold growth on plants reduced their photosynthetic efficiency, leading to formation of small sized bolls produced. The loss in seed cotton due to *P. solenopsis* infestation was estimated to be 40 to 50 per cent.

David *et al.* (2009) prepared bracket cages so as to culture the mealybug, *P. solenopsis* under screen house conditions. Different host plants were screened and

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Portulaca oleracea L. was found the best host plant among them as they are easy to propagate with stem cuttings and rooted quickly. They constructed the bracket cages by using Petri- plates, A4 sized transparent OHP sheets and triangular clips.

Arve *et al.* (2011) reported that mealybug nymphs and adults attacked all parts of cotton including their young shoots, flowers and fruits. They used to suck the plant sap and which resulted in growth malformation characterized by crinkling and curling of leaves. Infested flowers would drop and there was little production of bolls. Mealybugs produced honeydew which resulted in sooty mold production and reduced the photosynthetic capacity of plant.

Shafique *et al.* (2014) conducted experiments on *P. solenopsis* infested plants to study about the molecular and biochemical changes brought about by infestation, which revealed that mechanical injury made by the mealybug induced several cytological and physiological changes in host plants. Mealybug attack enhanced the production of cellulose, hemicellulose and lignin contents remarkably. It was also observed that the defensive biochemicals of cotton *i.e.* phenolics and terpenoids also increased significantly. Defensive enzymes like phenyl ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) showed direct relationship with the passing of time after mealybug feeding. Expression of thaumatin-like metallothionein and profilin genes was also enhanced with the insect herbivory.

2.4 Natural enemies of *Phenacoccus solenopsis*

Sharma (2007) conducted an experiment on parasitic efficiency of *Aenasius* sp. against *P. solenopsis* on okra at Hisar and observed that the per cent parasitization was 10, 20, 27, 45, 49, 57, 77 and 89 on 15th July, 30th July, 14th August, 29th august, 13th September, 28th September, 12th October and 26th October respectively and the mean parasitization of whole season was found to be 41.8 per cent.

Tanwar *et al.* (2008) reported large scale parasitism of *P. solenopsis* by *Aenasius* sp. (Hymenoptera : Encyrtidae) from three distant places Hisar,New Delhi and Parbhani in India. Survey conducted in five tehsils of Parbhani district of Maharashtra revealed the presence of cocoons of parasitoid, *Promuscidea unfasciativentris* Girault parasitizing *P. solenopsis.* This was found to be a solitary endoparasitoid causing mealybug to swell and change their colour to light brown. Developing larva will turn the mealybug into legless, brown, barrel shaped mummy with dark brown colour.

According to Nagrare *et al.* (2009) there should be minimum usage of synthetic insecticides, so that we could easily suppress the population of exotic mealybug like *P. solenopsis*. Ladybug beetle, *Cryptolaemus montrouzieri* Mulsant could be released on weeds and other preferred perennial host of mealybug prior to cotton cultivating season, which were found feeding on the mealybug voraciously. Amutha *et al.* (2009) observed natural parasitization of *P. solenospsis* as well as *Paracoccus marginatus* Williams & Granara de Willink by *Aenasius* sp. among several alternate hosts, where the maximum per cent of parasitization was found to be on *Abutilon indicum* (Link) Sweet (5 to 65%) followed by *P. hysterophorous* (5 to 30%). Gautham *et al.* (2009) reported that *Aenasius sp.* was as an effective parasitoid which parasitized 70 to 80 per cent of mealybugs in the field, where it was not recovered from any other locally existing mealybugs *viz., Nipaecoccus viridis* Newstead, *Rastrococcus iceryoides* Green, *Ferrisia virgata* (Cockerell) and *Planaococcus citri* (Risso). Hayat (2009) documented a new species of parasitoid *Aenasius bambawalei* Hayat from the mealybug, *P. solenopsis*. Natural parasitization of *P. solenopsis* was reported to be 8 to 26 per cent by two hymenopteran parasitoids *viz., Aenasius bambawalei* and another unidentified species (Saroja *et al.*, 2009). Muniappan (2009) reported that *A. bambawalei* was very effective in controlling *P. solenospsis* as it showed 60 per cent of parasitic efficiency in field level.

Gulsarbanu *et al.* (2009)investigated natural regulation of cotton mealybug populations by entomopathogens. A number of fungi such as, *Aspergillus clavatus* Desm., *Aspergillus oryzae* (Ahlburg) E. Cohn, *Aspergillus terreus* Thom and *Lecanicillium lecanii* R. Zare & W. Gams infected *P. solenopsis* in the field. Among them *L. lecanii* was found to be highly pathogenic to the mealybug under laboratory conditions. This was considered as the first report of natural occurrence of entomopathogenic fungi from mealybug.

Ram and Saini (2010) observed that, *Brumoides suturalis* (Fabricius) and *Nephus regularis* Sicard were the most dominant predators of *P. solenopsis*. Joshi *et al.* (2010) reported *Aenasius bambawalei* Hayat, *Anagyrus kamali* Moursi, *C. montrouzieri*, *Crysoperla carnea (Stephens)*, *Lecanicillium lecanii* and *Beauveria bassiana* (Bals. Criv.) Vuill as effective biocontrol agents against *P. solenopsis*.

Hanchinal *et al.* (2010) reported that spiders, green lace wings and coccinellids found to be the promising predators against the cotton mealybug, among them *C. carnea* was the most effective. Vijaya *et al.* (2010) observed that the extent of parasitization by *A. bambawalei* on mealybug, *P. solenopsis* ranged from 28.65 to 58.97 from the month of April to October, reaching its peak during September.

Arif *et al.* (2010) recorded several natural enemies of *P. solenopsis* such as, parasitoid *A. bambawalei* and predators, *B. suturalis*, *Scymnus coccivora* Ayyar, *Menochilus sexmaculata* (Fabricius) and spiders. Some other species were relatively less abundant such as, *Coccinella undecimpunctata* (Linnaeus), *Coccinella septempunctata* (Linnaeus), *Hyperaspis maindroni* Sicard, *Chrysoperla carnea* (Stephens), *Diadiplosis* sp. and *Geocoris* sp., while *Orius* sp. and *Campylomma* sp. were incidentally recorded on mealybug.

Tanwar *et al.* (2011) reported the parasitoid *Aenasius bambawalei* Hayat (Hemiptera : Encyrtidae) parasitizing on cotton mealybug intensively at many places,

viz., north zones of India, Punjab, Rajasthan, Madhya Pradesh and central zones, Maharashtra and Gujarat. It was found that the natural parasitization could reach more than 90 per cent at many locations. Sahito *et al.* (2011) conducted screening of several insecticides against cotton mealybug, *P. solenopsis* and its natural enemies which showed that, neem oil (repellent) to mealybug was the safest insecticide, followed by imidacloprid and prophenophos. Among the natural enemies, *A. bambawalei*, *B. suturalis* and *C. carnea* were found to be more tolerant to insecticides compared to others. Sangle (2011) found that the extent of parasitization by *A. bambawalei* on *P. solenopsis* ranged from 30.00 to 63.33 per cent with an average of 46.67 ± 2.03 per cent.

Khan *et al.* (2012) conducted an experiment to study the predatory potential of *Chrysoperla carnea* and *C. montrouzieri* larvae on different stages of *P. solenopsis* and reported that third instar nymph of *C. montrouzieri* devoured the highest mean number of first instar nymphs of *P. solenopsis*. *C. carnea* devoured relatively less number of *P. solenopsis* than *C. montrouzieri*. Both predators preferred first instar nymph of *P. solenopsis* over the second and third instar nymphal stages. Singh and Gandhi (2012) described the effect of different abiotic and biotic parameters on populations of *P. solenopsis* which revealed that mealybug population was positively correlated with temperature while that was negatively correlated with humidity. They also found there was a strong significant positive correlation with mealybug population with the percentage of parasitoids and coccinellid predators. They recorded the activity of parasitoids on cotton mealybug *P. solenopsis*, which started during the 43rd meteorological week and attained the peak population during 6th to 8th meteorological week.

Zhou *et al.* (2013) reported that the presence of red imported fire ants, *Solenopsis invicta* Buren reduced the population densities of both *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae) and ladybeetle, *M. sexmaculata*

(Coleoptera: Coccinellidae). Tending by the fire ants significantly reduced the survival of ladybeetle grub while there were no significant effects on survival of parasitoid wasp.

Maruthadurai and Singh (2015) reported 6.31 to 35.52 per cent parasitization of *P. solenopsis* by the encyrtid parasitoid, *A. bambawalei* (Hymenoptera: Encyrtidae). Zhou *et al.* (2015) showed that the *P. solenopsis* experienced a low per cent of parasitism on its host plant, *E. adenophorum*. The per cent parasitism by *A. bambawalei* and *M. sexmaculatus* was lower on *E. adenophorum* as compared to other host plants such as *G. hirsutum* and *H. rosa-sinensis* which in turn increased the population of *P. solenopsis*.

2.5 Management of *Phenacoccus solenopsis*

Bhosle *et al.* (2009) conducted field evaluation with 12 different insecticides and the study revealed that, acephate 70 SP followed by profenophos 50 EC and dichlorvos 76 EC was very effective for control of cotton mealybug. Nagrare *et al.* (2009), suggested that pigeon pea (*Cajanus cajan*) did not support the growth and multiplication of *P. solenopsis,* therefore cultivating border rows of pigeon pea as a barrier crop as well as strip crop after five to six rows of cotton may prevent mealybug infestation and spread. Rishi *et al.* (2009) reported that profenofos @ 1250 ml, monocrotophos @ 1250 ml, chlorpyriphos @ 3000 ml, quinalphos @ 2000 ml, acephate @ 2000 g, thiodicarb @ 625 g and carbaryl WP @ 2500 g/ha were very effective against *P. solenopsis*. Suresh *et al.* (2010) evaluated the effectiveness of insecticides against *P. solenopsis* by leaf dip method, which showed 100 percent reduction of *P. solenopsis* by chlorpyriphos followed by dichlorvos (90%), imidachloprid (89.99%), thiamethoxam (86.7%) and profenofos (80%). Joshi *et al.* (2010) observed that, field sanitation, uprooting of infested plants and dusting of plants with methyl parathion 2 per cent or spraying of chlorpyriphos 25 EC or

profenofos 25 EC or quinalphos 25 EC was very effective for the control of mealybug, *P. solenopsis*.

Lysandrou *et al.* (2012) evaluated the efficacy of insecticides, sulfoxaflor that act as nicotinic acetylcholine receptor (nAChR) competitive modulator and profenofos, an inhibitor of acetylcholinesterase enzyme on *P. solenopsis*. The experiment showed that, though sulfoxaflor were slower acting initially than profenofos, offered a very consistent and high level of activity. Profenofos overall, recorded the highest level of control and protection from the reinfestation of *P. solenopsis* in cotton plants, found to produce detrimental effects on plants such as the browning of leaves.

Afzal *et al.* (2015) reported a low level of resistance in *P. solenopsis* to acetamiprid and imidacloprid in the collected field populations compared to the control plot and research being carried out in author's laboratory suggested that the presence of monooxygenase and esterases was the major mechanism of resistance in *P. solenopsis* aiding in detoxification. The biological parameters of *P. solenopsis* were greatly affected by acetamiprid and the selected populations showed a relative fitness of 0.22, with significantly lower survival rate, pupal weight, fecundity, per cent hatching, biotic potential and mean relative growth rate, net reproductive rate, prolonged female and male nymphal durations, developmental time from egg to adult and male and female longevity compared with the control populations. Sanghi *et al.* (2015) conducted laboratory and field evaluation studies on effect of different insecticides on cotton mealybug during rainy season. Laboratory evaluations showed that highest mortality of mealybug was caused by thiamethoxam 25 WG which differed significantly from all other treatments. Lowest mortality was recorded with application of neem oil (1.5 %). Acephate (97%) DF showed most promising results in field conditions followed by thiamethoxam (25WG), imidacloprid 200 SL and (methomyl + thioacetiimidate) (40% SP).

According to Saddiq *et al.* (2015) a collection of *P. solenopsis* from different locations of Punjab, Pakistan with heavy usage of insecticides showed varied resistance towards insecticides. In their study, none to moderate level of resistance to nitenpyram, low to high level of resistance to acetamiprid and none to high resistance towards emamectin benzoate were observed. Very low to moderate levels of resistance were observed to insect growth regulators in tested populations of *P. solenopsis*. Sanghi *et al.* (2015) conducted experiments on efficacy of various insecticides on cotton mealybug *P. solenopsis* and concluded that prophenophos 2000 ml ha^{-1} excelled other insecticides and proved to be the most effective insecticide than others, followed by imidacloprid and dimethoate.

Singh and Kumar (2015) conducted behavioural bioassays under the laboratory conditions using semiochemicals obtained through n-hexane solvent volatile extract of *P. solenopsis*. Experiment revealed that the attractive index of male towards female was higher compared to other female mealybug. Further isolation of volatile extract using GC-MS, showed the presence of ester and terpene compound.

El- Zahi *et al.* (2016) reported that methomyl, chlorpyriphos, imidacloprid and thiamethoxam were very effective in controlling cotton mealybug, while the antifeedant compound (flonicamid) and avermectin derivative (emamectin benzoate) exhibited significantly lower activity. Mineral oil (KZ-oil) showed promising activity in controlling the mealybug population.

Nagrare *et al.* (2016) evaluated the efficacy of nineteen insecticide formulations from 10 different insecticide group against *P. solenopsis* Tinsley and its fortuitous parasitoid *A. bambawalei*. The results showed that thiamethoxam and profenophos have more than 70 per cent detrimental effect on both *P. solenopsis* and *A. bambawalei*. Thiodicarb was extremely toxic to *P. solenopsis* but relatively less

toxic to *A. bambawalei*, whereas spinosad was least toxic to *P. solenopsis* and highly toxic to *A. bambawalei*. Ismail *et al.* (2017) assessed the resistance evolution to chlorpyriphos and cross resistance to other insecticides in *P. solenopsis*. The experiment showed that *P. solenopsis* strain after 23 generations showed resistance to chlorpyriphos insecticide while those showed low level cross resistance to lamda cyhalothrin and very low level cross resistance to nitenpyram and profenofos.

2.6 Molecular characterization of *Phenacoccus solenopsis*

Hebert *et al.* (2003) proposed the concept of DNA barcoding as a reliable, more precise and most effective way of species identification. Although modern interactive versions represented a major advance, use of keys required high level expertise which often led to misidentification. To overcome this, they proposed the use of mitochondrial gene cytochrome *c* oxidase I (COI), which could serve as the core of a global bio-identification system for animals. They also suggested that species level assignments could also be obtained by creating comprehensive COI profiles. Asokan *et al.* (2011), reported that DNA barcoding could distinguish species which look alike, which could uncover the harmful organisms masquerading as harmless ones, which provided more accurate interpretations on biodiversity. Rebijith *et al.* (2012) suggested that DNA barcoding can identify the species from their different forms *viz.*, egg, larva and adult, and was a method for identification of species in a wide range of animal taxa, which used 5′ region of the mitochondria cytochrome c oxidase-I (CO-I). DNA barcoding was an easy, accurate and economical method of species discrimination, by developing species specific markers, which produced species specific amplicons for the targeted species. DNA barcoding also helped to identify invasive and cryptic species, haplotypes, biotypes and polymorphism among insects.

According to ICAC Recorder (2008) Central Institute for Cotton Research conducted DNA analysis of *P. solenopsis* colonies across India and results showed

that mealybug populations were identical and have little genetic diversity among the populations.

Dong *et al.* (2009) sequenced mitochondrial cytochrome oxidase COI (mtCO-I) gene of *Phenacoccuss solenopsis* and identified two divergent clades in the mealybug species based on the phylogenetic tree.

Abd-Rabou *et al.* (2012) combined molecular analysis of three DNA markers (28S-D2, cytochrome oxidase I and internal transcribed spacer 2) with morphological examination to identify different mealybug species collected from 40 mealybug populations of Egypt and France. Results revealed the presence of 17 different mealybug species including *P. solenopsis* from the populations collected from Egypt. Zhu *et al.* (2011) surveyed and sequenced 25 individual mealybug species to evaluate and discuss whether there exist cryptic lineages of *P. solenopsis*. They sequenced mtDNA COI gene sequences (694 bp) of individual mealybug from six different locations and analysed the existence of three haplotypes of *P. solenopsis*.

Singh *et al.* (2012) conducted molecular typing of cotton mealybug populations, *P. solenopsis* from different host and locations of Punjab. Adult mealybug collected from four host plants *i.e. G. hirsutum* – cotton, *A. esculentus*-okra, *Pennisetum glaucum* (L.) R. Br. - napier bajra and weed, *P. hysterophorous* and variability among populations was investigated through four different RAPD markers. Irrespective of the host plant, genetic similarity dendrogram was established. The study revealed that there was great possibility of development of biotypes which may differ in resistance to insecticides and host specificity.

Luo, *et al.* (2014) developed simple sequence repeats (SSR) genetic markers from the 28 120 unigenes of the transcriptome in the mealybug, *P. solenopsis* through a high- throughput method. The study showed that it was feasible to develop SSR markers by using *P. solenopsis* transcriptome and the primers developed in this study

provide foundation for the analysis of evolutionary biology, genetic diversity and invasion biology of the mealybug.

Thomas and Ramamurthy (2014) conducted experiments to study about the intraspecific variations in morphology of cotton mealybug populations, *P. solenopsis* from host plants *viz.,* cotton, hibiscus and okra. Genetic diversity studies using mitochondrial cytochrome oxidase I gene (mtCO-I) revealed that there was little variations in *P. solenopsis* collected from host plants of Asia, but there were very distinct separation between Asian and American populations.

Phylogenetic and homology difference analysis of *P. solenopsis* using mitochondrial cytochrome oxidase I gene, by Wu *et al.* (2015) found two haplotypes of the mealybug *P. solenopsis* from China and they showed that *P. solenopsis* mealybug from Southern China was probably closely related to populations from Pakisthan.

Ahmed *et al.* (2015) conducted genetic analysis for identifying *P. solenopsis* which revealed that *P. solenopsis* should be classified into two groups, one of which was found only in the United States and the other found only in Asia. The Asian group contained nine unique haplotypes, two among that invaded China, Pakisthan, India and Vietnam. The genetic analysis also provided some evidence on the relationship of mealybug with the parasitoid wasp, *A. bambawalei* Hayat.

Materials and methods

3. MATERIALS AND METHODS

Present study entitled, "Population dynamics, biology and management of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on okra" was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara between March 2016 and May 2017. The objectives of the investigation were to study the population dynamics, biology and management of *P. solenopsis* Tinsley and characterization of its endosymbionts. The details of the materials used and methods adopted for conducting various experiments based on the objectives set forth in this study are presented here under.

3.1 Survey and documentation of cotton mealybug, *Phenacoccus solenopsis* and their associated fauna

Purposive survey was carried out in five blocks of Thrissur district in Kerala. Among these blocks, different locations with okra cultivation were selected for the survey and GPS co-ordinates of the selected locations were recorded. The survey was carried out at monthly intervals during okra growing seasons between March 2016 and March 2017. Other host plants of the mealybug were also documented. Level of infestation was recorded based on a scale of zero to four (Anon., 2008) as follows.

0- No damage

1- Scattered appearance of mealybug

2- Fully infested on any one of the branch of the plant

3- Infestation of more than one branch or half portion of the plant

4- Heavy infestation on total plant

As there was no infestation of mealybug on okra during survey period, population studies of mealybug was conducted on four major host plants of *P.*

solenopsis viz., *Sida acuta* Burm.f., *Abutilon indicum* (Link) Sweet, *Hibiscus rosa-sinenensis* L. and *Amaranthus viridis* L. Number of mealybugs was counted using window count method from twenty randomly selected sampling units and population density of mealybug was calculated using the formula,

Table 1. Survey locations for collection of mealybug, *Phenacoccus solenopsis*

District	Block	Location	Geographical coordinates
Thrissur	Ollukkara	Madakkathara	10.5612° N & 76.2624°E
		Vellanikkara	10.3242°N & 76.1626°E
		Peechi	10.3137°N & 76.9126°E
		Pananchery	10.3326°N & 76.1826°E
		Kuttanellur	10.3016°N & 76.1625°E
		Valakkavu	10.3142°N & 76.1825°E
		Vettikkal	10.5319°N & 76.2523°E
	Anthikkad	Chazhur	10.4351°N & 76.1407°E
		Enamavu	10.3017°N & 76.0630°E
		Kanjani	10.2825°N & 76.6578°E
		Manalur	10.2944°N & 76.0636°E
	Cherppu	Avinisseri	10.4706°N & 76.2320°E
		Thaikkattusseri	10.4613°N & 76.2392°E
		Cherusseri	10.4481°N & 76.2323°E
		Ollur	10.2850°N & 76.1431°E
		Poochinnippadam	10.4324°N & 76.2045°E
		Anakkallu	10.4632°N & 76.2325°E
	Pazhayannur	Chelakkara	10.6941°N & 76.3464°E
		Elanadu	10.3995°N & 76.0630°E
	Puzhakkal	Poovani	10.3410°N & 76.1316°E
		Mulankunnathukavu	10.5958°N & 76.2076°E
		Kolazhy	10.5704°N & 76.2218°E

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$$\text{Population density} = \frac{\text{Total no. of individuals of the species from all sampling units (S)}}{\text{Total number of sampling units taken (Q)}}$$

Infested plants collected during survey period were observed for the presence of natural enemies *viz.,* predators, parasitoids and pathogens. Predators collected from the surveyed localities were identified at AINPAO, Department of Agricultural Entomology, College of Horticulture, KAU, Vellanikkara. Parasitoids were also separated after emergence and preserved in alcohol (70%). The preserved specimens were got identified at the Department of Zoology, Aligarh Muslim University, Uttar Pradesh.

3.2 Assessment of damage caused by *Phenacoccus solenopsis*

During laboratory studies, plant reaction was assessed based on leaf deformities (crinkling) caused by mealybug. Experiment was conducted on one week old as well as one month old okra plants. First instar, second instar, third instar and adult stages of mealybug were released in 2, 4, 6, 8 and 10 number per plant and damage score was calculated by leaf damage severity scale (Galanihe *et al.* 2011) after 96 h of release. Extent of leaf damage was recorded by visual scoring. Yield of okra plants were related with feeding of mealybugs at different stages.

Table 2. Leaf damage severity scale

Damage score	Extent of leaf crinkling (No. of leaves)
0	Undamaged (No Crinkling)
1	1-25 % leaves crinkled
2	26-50 % leaves crinkled
3	51- 75 % leaves crinkled
4	76-100 % leaves crinkled

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According to leaf damage severity scale there were five damage scores starting from 0 to 4.

Leaf crinkling per cent (crinkling %) was calculated using the given formula,

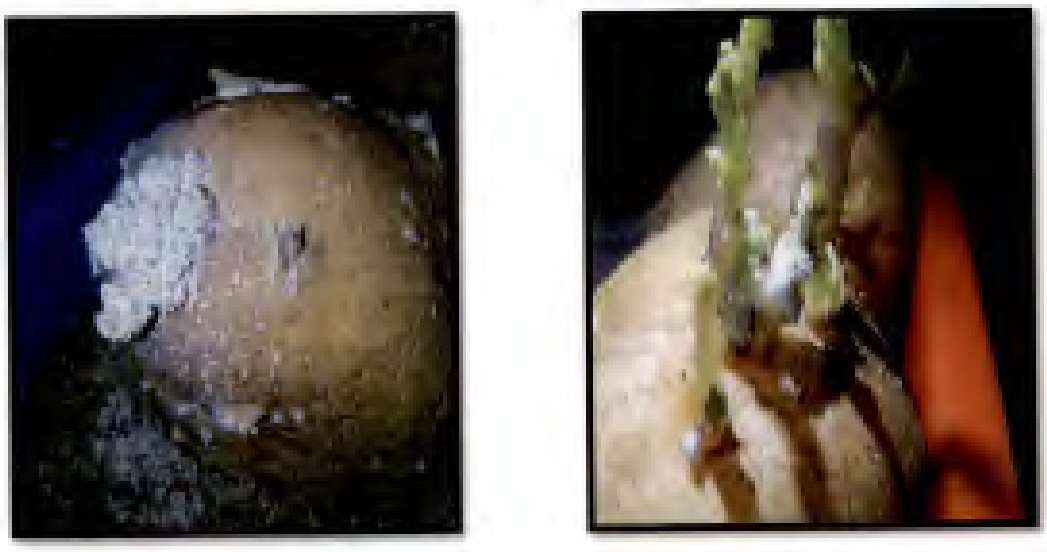
$$\text{Crinkling \%} = \left(\frac{\text{Number of crinkled leaves}}{\text{Total number of leaves (crinkled \& non crinkled)/plant}} \right) \times 100$$

3.3 Mass culturing of cotton mealybug, *Phenacoccus solenopsis*

To carry out studies on biology and pot culture experiments, mealybugs were reared on pumpkin as well as sprouted potatoes in the AINPAO laboratory, College of Horticulture, 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 wound found on pumpkin fruits were sealed by applying paraffin wax. Nymphs and adults collected from okra fields were released on pumpkin fruits (Plate 1).

As the mealybug population did not establish on pumpkin fruits, sprouted potatoes were used as substratum for the mealybug culture, hence selection of potato tubers was done very carefully. Medium to large sized sound sprouted potato tubers (150 to 200 g) were used for rearing of mealybugs. Care was taken while selection of the potato tubers, such that selected tubers were healthy, disease free and without any blackening around sprouts. Potato tubers were thoroughly washed in clean water to remove the adhered dirt and soil particles to prevent contamination by microorganisms. Later potato tubers were air dried and covered with slightly dampened sand and kept in cages for sprouting. Nymphs and adults of mealybug were released on sprouted potatoes using a camel brush (Plate 1).

Phenacoccus solenopsis on pumpkin



Phenacoccus solenopsis on sprouted potatoes

Plate 1. Rearing of *Phenacoccus solenopsis*

3.3.1 Biology of cotton mealybug

The study was conducted under ambient room conditions (28.81 ± 3.2 temperature and relative humidity of 75.5 ± 2.6 %). Twenty uniform first instar nymphs were collected from sprouted potato tubers and released on thoroughly washed and dried okra twigs of eight centimeter in length. Okra twigs were placed in Petri dishes lined with wet cotton and observed daily under microscope (Plate 2). Duration of each instar was confirmed based on the presence of moulted exuvia (Plate 3) of nymphs. Other parameters like pre - oviposition period, oviposition period, fecundity, post oviposition period adult longevity of male and female mealybugs, shape and colour of each stage were recorded. Morphometric observations like length and width of mealybugs were also taken from 20 randomly collected mealybugs.Duration of development of different stages of *P. solenopsis*, adult longevity, pre-oviposition, oviposition and post- oviposition periods, number of nymphs per female 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.

3.4 Preparation of spore suspension using haemocytometer

Cultures of the two entomopathogenic fungi, *Paecilomyces lilacinus (Purpureocillium lilacinum)* (Thom) Samson and *Lecanicillium lecanii* (Zimm.) were obtained from BRS Kannara and AICRP on BCCP & W, Kerala Agricultural University, respectively. The fungal cultures were crushed in wearing blender and made into liquid 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 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).

Plate 2. Biology of mealybug, *Phenacoccus solenopsis* on okra twigs

Plate 3. Exuvia of mealybug, *Phenacoccus solenopsis*

$$\text{Number of spores ml}^{-1} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where,

X = Number of squares counted from small squares

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 number of spores, both the cultures were diluted to obtain 1×10^7 spores ml^{-1} and 1×10^8 spores ml^{-1}concentrations from the higher concentration of 1×10^9 spores ml^{-1} for conducting the pot culture evaluation studies.

3.4.1 Evaluation of entomopathogenic fungi, botanicals and chemical insecticides under pot culture experiment

Entomopathogenic fungi *viz., P. lilacinus* and *L. lecanii*, botanicals *viz.*, neem oil soap and NSKE, chemicals insecticides *viz.*, buprofezin (25 % EC) and thiamethoxam (25 WG) were used to conduct the pot culture experiment. Okra seeds (variety: Arka Anamika) were procured from Central Nursery, Kerala Agricultural University, Vellanikkara. The experiment was conducted in completely randomized block design (CRD) with eleven treatments and three replications (four plants per replication) (Plate 4). Each treatment was blocked from one another by using wire net for preventing migration of mealybugs within the treatments. Third instar nymphs

Pot culture experiment with okra plants

Spraying of okra plants with pneumatic hand sprayer

Plate 4. Layout of pot culture experiment and application of treatments

were released on 30 days okra plants at the rate of 40 nymphs per plant with the help of camel hair brush. Two weeks after the release of nymphs, pre-count of mealybug was taken prior to the application of treatments. The treatments were applied using pneumatic hand sprayer (Plate 4).

Table 3. Evaluation of treatments on *Phenacoccus solenopsis* in pot culture experiment

Treatments	Frequency of application
T_1 : *Paecilomyces lilacinus* @ 1×10^7 spores ml^{-1}	Two sprays at 7 days interval
T_2 : *P. lilacinus* @ 1×10^8 spores ml^{-1}	Two sprays at 7 days interval
T_3 : *P. lilacinus* @ 1×10^9 spores ml^{-1}	Two sprays at 7 days interval
T_4 : *Lecanicillium lecanii* @ 1×10^7 spores ml^{-1}	Two sprays at 7 days interval
T_5 : *L. lecanii* @ 1×10^8 spores ml^{-1}	Two sprays at 7 days interval
T_6 : *L. lecanii* @ 1×10^9 spores ml^{-1}	Two sprays at 7 days interval
T_7 : Neem oil soap @ 2 %	Two sprays at 7 days interval
T_8 : NSKE @ 5%	Two sprays at 7 days interval
T_9 : Buprofezin @ 250 a.i. ha^{-1}	Single spray
T_{10} : Thiamethoxam @ 25 a.i. ha^{-1}	Single spray
T_{11} : Untreated control	

3.4.2 Observations

The number of mealybugs was recorded before and after the application of each treatment. Count of the mealybugs on each plant was taken every third day after the application of treatments. The dead mealybugs were collected and placed in Petri dish lined with a moist filter paper and were observed for mycelial growth. The mean population of mealybugs of both pre and post count was analysed by Analysis of Covariance (ANOCOVA). Reduction in number of mealybugs over control was

analysed by ANOCOVA and means were separated by Duncan's Multiple Range Test (DMRT).

3.5 Molecular characterization of endosymbionts of *Phenacoccus solenopsis*

3.5.1 Sample collection and storage of DNA

Mealybug samples were collected from infested okra field for the extraction of DNA. Collected samples were preserved in 95 per cent ethanol and stored at -20°C.

3.5.2 Isolation of metagenomic DNA from mealybug, *Phenacoccus solenopsis*

Mealybugs were surface sterilized in de-ionized water and was then blotted carefully to remove the moisture. They were grounded in 1.5 ml Eppendorf® tube with sterilized micropistle with addition of 1 ml pre-warmed (65°C for 10 min) extraction buffer [SDS (0.5 %), 200mM Tris-HCl (P^H 8), 25mM EDTA (p^H 8), 250mM NaCl] and vortexed for 5 min. The slurry was transferred to centrifuge tube and incubated at room temperature for 1h, later centrifuged at 10,000 rpm for 8 min and supernatant was collected. An equal volume of phenol: chloroform isoamyl alcohol (25 : 24 : 1) was added and centrifuged at 9000 rpm for 23 min at 4°C. Supernatant was collected and DNA was precipitated with equal volume of ice cold isopropanol and incubated at room temperature for 30 min. Later the mixture was centrifuged at 10,000 rpm at room temperature for 8 min. Supernatant was discarded and 600 µl of ethanol was added. DNA pellet was washed by centrifugation at 10,000 rpm for 12 min. DNA pellet was air dried and dissolved in 25µl distilled water and stored in deep freezer for future use. The quality of DNA was assessed by one per cent agarose electrophoresis.

3.5.3 Polymerase chain reaction with metagenomic DNA

From the DNA sample a 1.5 KB region of bacterial 16S rDNA genome was amplified by using the primers (**F:** 16S rDNA – GAGTTTGATCCTGGCTCAG, R: 16S rDNA - ACGGCTACCTTGTTACGACTT) in Veriti Thermal Cycler (Applied Biosystems®). The PCR reaction was carried out using , 1µl template DNA, 0.5 µl of forward and reverse primers, 1.5µl of 10mM dNTP (Genei®), 0.4 µl of Taq DNA polymerase (Genei®), 2µl of Taq DNA buffer B (Genei®), 1.5µl of $MgCl_2$ and 12.6µl of Millipore® water. The PCR conditions were programmed as, lid temperature 98°C, initial denaturation 94°C for 4 min, 35 cycles each of denaturation 94°C for 30 seconds, primer annealing 45°C for 1 min and primer extension 72°C for 2 min, followed by 10 min extension at 72°C and storage at 4°C. The amplified PCR product was run on agarose gel electrophoresis (1.2%) and the product was sequenced at Sci Genome labs, Cochin. Total raw sequences obtained from sequencer were checked for quality parameters viz., base quality parameters, base parameters, base composition distribution and GC data. After trimming the unwanted sequences from original paired- end data, a consensus V3 region sequence was constructed using Clustal Omega program. Further we applied multiple filters and the highest quality V3 region was taken for downstream analyses. Downstream analysis of sequences was carried out by MG- RAST (Metagenomics Rapid Annotations using Subsystems Technology) program and abundance of endosymbionts was calculated and pie diagrams were constructed.

3.5.4 Isolation of genomic DNA from mealybug, *Phenacoccus solenopsis*

Mealybugs were surface sterilized in de-ionized water and was then wiped carefully to remove the moisture. The total genomic DNA of mealybug was isolated using CTAB extraction method. Briefly, mealybugs were crushed in 1.5 ml Eppendorf tube with sterilized micropistle with addition of 0.9 ml pre-warmed (65°C for 10 min) modified CTAB extraction buffer [CTAB (2%), 1M Tris-HCl (p^H 8),

0.5M EDTA (p^H 8), 5M NaCl, distilled water]. The slurry was transferred to sterile centrifuge tube and incubated at 65°C for 90 min with gentle shaking. An equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was collected in fresh 1.5 ml eppendorf tube with addition of chloroform: isoamyl alcohol, vortexed and centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was collected, added equal volume of chloroform: isoamyl alcohol and mixed by gentle inversion. 3M sodium acetate (P^H 5.8), 30 µl was added and incubated at -20°C for 1 hour, centrifuged at 13,000 rpm for 10 min at 4°C. Supernatant was removed and DNA was washed with ethanol (70%) by centrifugation at 13,000 rpm for 10 min at 4°C. The DNA pellet was air dried and and dissolved in 25µl distilled water and stored in deep freezer for future use. The quality of DNA was assessed by one per cent agarose electrophoresis.

3.5.5 Polymerase chain reaction with DNA barcode primer and sequencing

Good quality genomic DNA (50ng/µl) isolated from mealybug was used for DNA barcoding. The universal barcode primer [Hebert *et al.,* 2003] specific to mitochondrial cytochrome oxidase I (mtCOI) was used for PCR amplification. The mtCOI region was amplified by polymerase chain reaction from genomic DNA using the universal barcode primers (C1-J-2183F–CAACATTTATTTTGATTTTTTGG, C1-N-2568–R- GCWACWACRTAATAKGTATCATG) in Veriti Thermal Cycler (Applied Biosystems®). The PCR reaction was carried out using, 0.6µl template DNA (50ng), 0.6µl forward and reverse primers, 1.8µl of 10mM dNTP (Genei®), 0.4 µl of Taq DNA polymerase (Genei®), 2µl of Taq DNA buffer B (Genei®), 1.8µl of $MgCl_2$ and 12.2µl of Millipore® water. The PCR conditions were programmed as, lid temperature 98°C, initial denaturation 94°C for 4 min, 35 cycles each of denaturation 94°C for 30 seconds, primer annealing 45°C for 1 min and primer extension 72°C for 2 min, followed by 10 min extension at 72°C and storage at 4°C. The amplified PCR product was run on agarose gel electrophoresis (1%) and the product was sequenced

at Sci Genome labs, Cochin. The sequences generated from the study was analyzed for sequence homology using nucleotide BLAST at NCBI, submitted to BankIt, GenBank and the accession number was generated. A phylogenetic tree was constructed using available accessions using Clustal Omega. Later the specimen details *viz.,* sequences, primers, images and traces were uploaded at BOLD and barcode of *P. solenopsis* was generated.

Results

4. RESULTS

The results obtained from the study "Population dynamics, biology and management of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on okra are presented in this chapter.

4.1 Survey and documentation of cotton mealybug, *Phenacoccus solenopsis* and their associated fauna

A preliminary survey was conducted during March 2016in okra cultivating areas of Thrissur district to collect the mealybug, *P. solenopsis* on okra. Five blocks were surveyed in Thrisur district. However no infestation of mealybug was found in okra fields (Plate 5) (Table 4). Survey was continued till March 2017 and 44 host plants of mealybug were recorded with heavy infestation (Table 5). Among them, weed hosts were more compared to vegetables and ornamentals (Plate 6). *Abutilon indicum* (Link) Sweet, *Amaranthus viridis* L., *Sida acuta* Burm. f. were recorded as the major weed hosts which found infested with the mealybug throughout the year.Vegetable crops infested with the mealybug were chilli, brinjal, tomato, cucumber, spinach *etc.* Okra was also found infested with *P. solenopsis* at homestead gardens. However, infestation was very less compared to that of ornamentals such as, China rose, Mexican marigold, sulphur cosmos *etc.* As the mealybug infested okra fields were scarce, population of *P. solenopsis* was recorded on four major hosts *viz.*, *Sida acuta* Burm. f., *Abutilon indicum* L., *Hibiscus rosa-sinensis* L. and *Amaranthus viridis* L. at selected locations in Thrissur *viz.,* Vellanikkara, Madakkathara, Mannuthy, Peechi and Pananchery from March 2016 to March 2017 (Table 6). The observations and scoring of selected host plants showed that, populations of mealybug decreased during monsoon season compared to that of summer and post – monsoon season. *S. acuta* and *H. rosa- sinensis* was found infested with the mealybug throughout the year, while other two host plants showed higher infestation levels during summer season. Natural enemy fauna associated with the mealybug was

Plate 5. Survey locations of okra fields for *Phenacoccus solenopsis* infestation

recorded from the five selected locations (Table 7), which showed the presence of four parasitoids and a predator. Species diversity of parasitoids was more at Pananchery and Peechi compared to other locations. Parasitoidswere identified as*Aenasius arizonenesis* (Girault) (Hymenoptera: Encyrtidae) (Plate 7), *Anicetus* sp. (Hymenoptera: Encyrtidae) (Plate 8), *Myiocnema comperei* Ashmead (Hymenoptera: Aphelinidae) (Plate 8)and *Prochiloneurus* spp. (Hymenoptera : Encyrtidae) (Plate 8) identified by Dr. Mohammad Hayat of Aligarh Muslim University. *A. arizonensis* was the most common parasitoid observed along with *P. solenopsis*. Larval predator collected from mealybugs was reared and identified as *Spalgis epius*(Westwood) (Lepidoptera: Lycaenidae) (Plate 9) from Department of Agricultural Entomology, KAU, Vellanikkara. Abundance of natural enemy fauna from each location were calculated. During summer season, total number of natural enemies found was 143, and among them *A. arizonensis* dominated the other natural enemies (Table 8). Natural enemy populations decreased during monsoon season, where *S. epius* and *A. arizonensis* were only observed in five locations (Table 9). Populations of natural enemies increased during post- monsoon season, where the relative abundance of *A. arizonensis* was found the highest (Table 10) than summer and monsoon seasons.

Table 4. Preliminary survey on infestation of mealybug, *Phenacoccus solenopsis* on okra in Thrissur

Districts	Blocks	Locations	Number of fields visited	Presence or absence of cotton mealybug infestation on okra
Thrissur	Ollukkara	Madakkathara	3	Absent
		Vellanikkara	5	Absent
		Peechi	1	Absent
		Pananchery	2	Absent
		Kuttanellur	2	Absent
		Valakkavu	1	Absent
		Vettikkal	3	Absent
	Anthikkad	Chazhur	4	Absent
		Enamavu	2	Absent
		Kanjani	3	Absent
		Manalur	2	Absent
	Cherppu	Avinisseri	2	Absent
		Thaikkattusseri	5	Absent
		Cherusseri	4	Absent
		Ollur	2	Absent
		Poochinnippadam	1	Absent
		Anakkallu	1	Absent
	Pazhayannur	Chelakkara	3	Absent
		Elanadu	1	Absent
	Puzhakkal	Poovani	2	Absent
		Mulankunnathukavu	3	Absent
		Kolazhy	1	Absent

Phenacoccus solenopsis on chilli

Phenacoccus solenopsis on brinjal

Phenacoccus solenopsis on wire weed

Plate 6. Host plants of mealybug, *Phenacoccus solenopsis*

Table 5. Host palnts of *Phenacoccus solenopsis* recorded during survey

	Common Name	Botanical Name
A	**Weed hosts**	
1	Indian mallow	*Abutilon indicum*
2	Mountain knotgrass	*Aerva lanata*
3	Goatweed	*Ageratum conyzoids*
4	Green amaranth	*Amaranthus viridis*
5	Spreading hogweed	*Boerhavia diffusa*
6	Tindora	*Coccinia indica*
7	Sacred datura	*Datura wrightii*
8	Beggar weed	*Desmodium tortuosum*
9	Bell weed	*Dipteracanthus prostratus*
10	Lilac tasselflower	*Emilia sonchifolia*
11	Siam weed	*Eupatorium odoratum*
12	Asthma plant	*Euphorbia hirta*
13	South American mock vervain	*Glandularia pulchella*
14	Globe amaranth	*Gomphrena globosa*
15	Levant cotton	*Gossypium herbaceum*
16	True indigo	*Indigofera tinctoria*
17	Willow-leaved Justicia	*Justicia gendarussa*
18	Spanish flag	*Lantana camara*
19	Creeping oxeyes	*Wedelia trilobata*
20	Elegant zinnia	*Zinnia elegans*
21	Climping hemp vine	*Mikania micrantha*
22	Bush passion fruit	*Passiflora foetida*
23	Indian beech tree	*Pongamia pinnata*
24	Snapdragon root	*Ruellia tuberosa*
25	Licorice weed	*Scoparia dulsis*
26	Wireweed	*Sida acuta*
27	Heart-leaf sida	*Sida cordata*
28	Flannel weed	*Sida cordifolia*
29	Snapdragon root	*Ruellia tuberosa*
30	Canadian goldenrod	*Solidago canadensis*
31	Nodeweed	*Synedrella nodiflora*
32	Tridax daisy	*Tridax procumbens*
B	**Vegetables and flowering plants**	
1	Chilli	*Capsicum annuum*
2	Winter melon	*Benincasa hispida*
3	Sulphur cosmos	*Cosmos sulphureus*

4	Cucumber	*Cucumis sativus*
5	China rose	*Hibiscus rosa-sinensis*
6	Tomato	*Lycopersicum esculentum*
7	Cassava	*Manihot esculenta*
8	Guava	*Psidium guajava*
9	Brinjal	*Solanum melongena*
10	Okra	*Abelmoschus esculentus* L. Moench
11	Spinach	*Spinasia oleraceae*
12	Mexican marigold	*Tagetes erecta*

Table 6. Severity scoring on *Sida acuta, Hibiscus rosa-sinensis, Abutilon indicum* and *Amaranthus viridis* infested with *Phenacoccus solenopsis*

Month	Leaf damage severity															
	Vellanikkara				Thottappady			Mannuthy			Peechi			Pananchery		
	Sa	Hr	Ai	Av	Sa	Hr	Ai	Sa	Hr	Ai	Sa	Hr	Ai	Sa	Hr	Ai
Mar 16	2	2	2	1	2	2	1	2	2	2	2	2	2	2	3	2
Apr 16	3	2	2	1	2	2	1	2	2	2	2	2	3	2	3	2
May 16	3	2	2	1	2	3	1	3	2	2	2	3	3	3	3	3
Jun 16	3	2	1	-	2	3	1	3	2	1	2	3	3	3	3	2
Jul 16	3	2	1	-	2	3	-	3	3	1	1	2	-	3	2	-
Aug 16	2	2	-	-	2	-	-	2	2	-	1	2	-	2	2	-
Sep 16	2	-	-	-	2	-	-	2	2	-	1	2	-	2	2	-
Oct 16	2	-	-	-	2	-	-	2	2	-	1	2	-	2	2	-
Nov 16	3	-	-	-	3	2	-	2	2	-	3	2	-	2		-
Dec 16	3	-	-	-	3	3	-	3	3	-	3	2	-	2	3	-
Jan 17	4	1	-	-	3	3	-	3	3	1	3	3	2	3	2	-
Feb 17	3	1	-	-	3	3	2	3	3	1	3	3	3	3	3	-
Mar 17	3	2	-	-	3	3	1	3	3	2	3	3	3	3	3	-

Sa- *Sida acuta*, Hr- *Hibiscus rosa – sinensis*, Ai- *Abutilon indicum*, Av- *Amaranthus viridis*

A. arizonensis (Male)

A. arizonensis (Female)

Plate 7. Parasitoid, *Aenasius arizonensis*

Anicetus sp. (35X)

Myiocnema comperei (35 X)

Prochiloneurus spp. (35 X)

Plate 8. Parasitoids of mealybug, *Phenacoccus solenopsis*

A. Larva

B. Pupa

C. Adult

Plate 9. Developmental stages of *Spalgis epius*

Table 7. Distribution of natural enemies in selected localities of Thrissur district

Natural enemies		Locations				
		Vellanikkara	Thottappady	Mannuthy	Peechi	Pananchery
Mar'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Apr'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
May'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae, Pr
Jun'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae, An, My	Ae
Jul'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Jul'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Aug'16	Pr	Sp	Sp	Sp	-	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Sep'16	Pr	Sp	Sp	Sp	-	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Oct'16	Pr	Sp	Sp	Sp	-	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Nov'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Dec'16	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae
Jan'17	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae, My, An
Feb'17	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae, My
Mar'17	Pr	Sp	Sp	Sp	Sp	Sp
	Pa	Ae	Ae	Ae	Ae	Ae

Pr- Predator, Pa- Parasitoid, Sp- *Spalgis epius*, Ae- *Aenasius arizonensis*, An- *Anicetus* sp., My- *Myiocnema comperei*, Pr- *Prochiloneurus* spp.

Table 8. Relative abundance of parasitoids and predators in selected localities of Thrissur district during summer season

Locations	Number of natural enemies	Number of predator	Number of parasitoids				Relative abundance of predator (%)	Relative abundance of parasitoids (%)			
		Sp	Ae	An	My	Pr	Sp	Ae	An	My	Pr
Vellanikkara	38	13	25	-	-	-	34.2	65.7	-	-	-
Thottappady	28	8	20	-	-	-	28.5	71.4	-	-	-
Mannuthy	21	14	7	-	-	-	66.6	33.3	-	-	-
Peechi	14	4	9	-	-	1	28.5	64.2	-	-	7.1
Pananchery	42	13	26	1	2	-	30.9	61.9	2.3	4.7	-
Total	143	52	87	1	2	1	36.6	60.8	0.6	1.3	0.6

Sp- *Spalgis epius*, Ae- *Aenasius arizonensis*, An- *Anicetus* sp., My- *Myiocnema comperei*, Pr- *Prochiloneurus* spp.

Table 9. Relative abundance of parasitoids and predators in selected localities of Thrissur district during monsoon season

Locations	Number of natural enemies	Number of predator	Number of parasitoids				Relative abundance of predators (%)	Relative abundance of parasitoids (%)			
		Sp	Ae	An	My	Pr	Sp	Ae	An	My	Pr
Vellanikkara	17	7	10	-	-	-	41.2	58.8	-	-	-
Thottappady	13	6	7	-	-	-	46.2	53.8	-	-	-
Mannuthy	8	6	2	-	-	-	75.0	25.0	-	-	-
Peechi	10	-	10	-	-	-	100	-	-	-	-
Pananchery	18	7	11	-	-	-	38.8	61.2	-	-	-
Total	66	26	40	0	0	0	39.9	61.0	0	0	0

Sp- *Spalgis epius*, Ae- *Aenasius arizonensis*, An- *Anicetus* sp., My- *Myiocnema comperei*, Pr- *Prochiloneurus* spp.

Table 10. Relative abundance of parasitoids and predators in selected localities of Thrissur district during post-monsoon season

Locations	Number of natural enemies	Number of predator	Number of parasitoids				Relative abundance of predators (%)	Relative abundance of parasitoids (%)			
		Sp	Ae	An	My	Pr	Sp	Ae	An	My	Pr
Vellanikkara	29	2	27	-	-	-	6.89	93.10	-	-	-
Thottappady	36	11	25	-	-	-	30.50	69.40	-	-	-
Mannuthy	16	4	10	-	-	-	25.00	62.50	-	-	-
Peechi	12	8	4	-	-	-	66.60	33.33	-	-	-
Pananchery	19	2	14	1	2	-	10.50	73.62	5.20	10.50	-
Total	112	27	80	1	2	0	24.12	71.40	0.81	1.71	0.00

Sp- *Spalgis epius*, Ae- *Aenasius arizonensis*, An- *Anicetus* sp., My- *Myiocnema comperei*, Pr- *Prochiloneurus* spp.

4.1.1 Ants associated with *Phenacoccus solenopsis*

One species of ant collected from the mealybug infested okra plants was identified by Dr. K. A. Karmaly, Taxonomist, Dept. of zoology, St. Xavier's College for Women, Aluva, Ernakulam, as *Anoplolepis gracilipes* (Smith) (Plate 10).

4.2 Assessment of damage caused by *Phenacoccus solenopsis*

Leaf damage severity scale was developed using the damage scale as explained in 3.2 (Table 2). The observations were recorded 96 h after release of mealybug.

No leaf crinkling symptom was recorded on one week old plants with pest load of 2, 4 and 6 number of first instar nymphs, thus the leaf crinkling score was '0' (Table 11). Crinkling symptoms appeared when the pest load was increased to eight and ten first instar nymphs per plant (9.42 and 19.00 %, respectively) with the leaf crinkling score '1'. With the second instar nymphs, leaf crinkling symptoms appeared on the okra plants, when the pest load was 6 nymphs per plant (4.70 % with a score of '1'). Per cent leaf crinkling increased along with the increase in pest load of 8 and 10 number of second instar nymphs per plant (9.53 and 28.5 % with a score of '1' and '2' respectively). Third instar nymphs showed crinkling symptoms with release of six nymphs per okra plant (4.7 % with a score of '1'), while per cent leaf crinkling increased with increase in pest load from 8 to 10 third instar nymphs per plant (23.80 and 38.10 % with a score of '1' and '2' respectively). With the adult mealybugs, crinkling symptoms appeared with a pest load of 8 and 10 number of mealybugs per okra plant (4.7 % with a score of '1').

No leaf crinkling symptoms were produced by one month old okra plants with a pest load of 2, 4, 6, 8 and 10 number of mealybugs, thus the leaf crinkling score given was '0' (Table 12). With the second instar nymphs, leaf crinkling symptoms appeared with a pest load of 8 and 10 number of mealybugs per plant (4.70 and 9.50

Plate 10. Ant species associated with *Phenacoccus solenopsis*

***Anoplolepis gracilipes* (Smith)**

% with a score of '1') While they showed a crinkling percentage of about 4.7 to 9.5 per cent (score'1'). Leaf crinkling symptoms appeared on plant with the release of 8 and 10 number of third instar nymphs per plant (19.00 and 23.80 % with a score of '1'). No leaf crinkling symptoms were appeared on plant with the release of adult mealybugs (score '0').

Yield of okra plants were taken after five weeks and two weeks of release of mealybugs on one week old and one month old okra plants respectively. It was found that, yield of non-infested okra plants was higher than that of mealybug infested okra plants. Infestation at early stages of okra caused reduced yield as compared to that of one month old okra plants (Table 13).

Table 11.Assessment of plant reaction to *Phenacoccus solenopsis* on one week old okra plant

Number of mealybugs released per plant	Leaf crinkling*							
	I instar		II instar		III instar		Adult	
	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
6	0	0	4.7	1	4.7	1	0	0
8	9.42	1	9.53	1	23.8	1	4.7	1
10	19	1	28.5	2	38.1	2	4.7	1

*Mean of three observation

Table 12.Assessment of plant reaction to *Phenacoccus solenopsis* on one month old okra plant

Number of mealybugs released per plant	Leaf crinkling*							
	I instar		II instar		III instar		Adult	
	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score	Crinkling (%)	Score
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
8	0	0	4.7	1	19	1	0	0
10	0	0	9.5	1	23.8	1	0	0

*Mean of three observations

Table 13. Effect of *Phenacoccus solenopsis* infestation on yield in okra

Treatments	*Yield	
	One week old plant	One month old plant
I instar	0.20	0.22
II instar	0.16	0.21
III instar	0.14	0.22
Adult mealybug	0.24	0.28
Control	0.32	0.31

* Yield (g) per fifteen okra plants

4.3Biology of *Phenacoccus solenopsis*

The biology of *P. solenopsis* was carried out in laboratory of All India Network Project on Agricultural Ornithology, College of Horticulture, KAU, Vellanikkara (Table 14). Twenty first instar crawlers of the same age were introduced on okra twigs (5 cm in length). The study showed that there were three nymphal instars and an adult stage for the female mealybug, whereas male possessed four nymphal instars including an additional pupal stage prior to its molt into adult stage. In the present study, parthenogenesis and ovo-viviparity dominated over oviparous mode of reproduction. Populationof male mealybug was very less compared to female mealybugs.Total nymphal period in female mealybug ranged from 12.5 to 18.5 days with an average of 15.3 ± 1.74 days whereas male mealybug showed slightly longer nymphal period ranged from 16.5 to 23.75 with an average of 18.88 ± 1.6 days.

4.3.1 First instar nymph

The first instar nymphs were very active and fast moving. They were green-yellowish and had dorso-ventrally flattened body with six segmented antenna (Plate 11). They were found aggregating under the thread like structure produced by the adult female mealybug. The first instar nymphs were devoid of wax coating over their body (Plate 12). The duration of first instar nymph ranged from 3 to 6.5 days with an average of 4.27 ± 0.84 days (Table 14). The length and width of crawlers varied from0.415 mm to 0.426 mm and 0.212 mm to 0.218 mm with an average of 0.42 ± 0.003 mm and 0.215 ± 0.001 mm (Table 15), respectively.

4.3.2 Second instar nymph

The second instar nymphs were partially covered with wax covering and were similar in their morphological features to that of the first instar nymphs having yellowish flattened body (Plate 12) and possessing six segmented antennae (Plate 11). Duration of the nymphs lasted from 5 to 8.5 days with an average of 6.67 ± 0.97 days (Table 14). The length and width of the nymphs ranged from 0.63 mm to 0.85 mm with an average of 0.74 ± .06 mm and 0.32 mm to 0.43 mm with an average of 0.38 ± 0.03 mm (Table 15).

4.3.3 Third instar nymph

Nymphs were yellowish with flattened body. Body of the third instar nymphs were fully covered with waxy covering (Plate 12) and they had seven segmented antennae (Plate 11). Duration of third instar nymphs ranged between 3 to 8.5 days with an average of 4.8 ± 1.33 days (Table 14). The length of third instar nymph ranged from 1.13 mm to 1.83 mm with an average of 1.42 ± 0.25 mm. The width of mealybug ranged from 0.57 mm to 1.01 mm with an average of 0.71 ± 0.15 mm (Table 15).

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a. First instar

b. Second instar

c. Third instar

d. Adult

Plate 11. Antennal segments in different instars of *Phenacoccus solenopsis*

Table 14.Biology of *Phenacoccus solenopsis*

Stage	Duration (Days)	
	*Mean	Range
Development period		
First instar	4.27 ± 0.84	3.00 - 6.50
Second instar	6.67 ± 0.97	5.00 - 8.50
Third instar	4.80 ± 1.33	3.00 - 8.50
Pupa	6.80 ± 1.06	5.25 – 9.00
Adult		
Male	1.70 ± 0.55	0.75 – 3.00
Female	38.75 ± 2.80	34.00 - 44.50
Reprodutive period		
Incubation period‡	81.45 ± 40.00	30.00 – 165.00
Pre-oviposition period	7.00 ± 1.50	5.00 - 9.50
Oviposition period	15.00 ± 2.07	11.50 – 20.00
Post-oviposition period	1.35 ± 0.58	0.50 – 2.00
Number of crawlers/ female (Ovo - viviparity)	171.70 ± 28.15	125 – 218
Number of crawlers/ female (Parthenogenesis)	93.45 ± 7.63	76.00 – 105.00
Total life cycle		
Male	20.43 ± 1.76	16.75 – 25.25
Female	38.78 ± 2.86	34.00 – 44.50

*Mean of 20 observations

‡ Incubation period in minutes

4.3.4 Male pupal instar

Male mealybug possessed an additional pupal stage. Pre- pupal period after second instar lasted for 1 to 2 days with an average of 1.4 ± .47 days. Pupal duration in male ranged from 5.25 to 9 days with an average of 6.8 ± 1.06 days (Table 14). The length of the male pupa ranged from 1.83 mm to 1.91 mm with an average of 1.87 ± 0.02 mm. The width of pupa ranged from 0.58 mm to 0.62 mm with an average of 0.59 ± 0.01 mm (Table 15) (Plate 13).

4.3.5 Adult female

Adult female had a convex yellowish body which was covered with thick waxy coating (Plate 12) and possessed nine segmented antennae (Plate 11). Adult lived for about 34 to 44.5 days with an average of 38.75 ± 2.8 days (Table 14). The length of adult female mealybug ranged from 3.12 mm to 3.7 mm with a mean of 3.5 ± 0.20 mm. Adult mealybug was observed to have a width ranged from 1.8 mm to 2.02 mm with an average of 1.92 ± 0.08 mm (Table 15).

Adults were ovo-viviparous and reproduced parthenogenetically under laboratory conditions. Adult female mealybug produced a thread like structure called ovisac, within which the first instar nymphs were found.

The pre- oviposition period of the female mealybug lasted for about 5 to 9.5 days with an average of 7 ± 1.5 days. The duration of oviposition period ranged from 11.5 to 20 days with an average of 15 ± 2.07 days, while the post- oviposition period lasted for 0.5 to 2 days with an average of 1.35 ± 0.58 days (Table 14). The total number of crawlers produced by adult mealybug ranged from 125 to 218 with an average of 171 ± 28.15 (Table 14). Female to male ratio was found to be 1: 0.03.

a. First instar nymph

b. Second instar nymph

c. Third instar nymph

d. Adult female

Plate 12. Developmental stages of *Phenacoccus solenopsis* (Female)

a. First instar nymph

b. Second instar nymph

c. Male pupa

d. Adult male

Plate 13. Developmental stages of *Phenacoccus solenopsis* male

4.3.6 Adult male

Adult male mealybug was winged with whitish body (Plate 13). Duration of adult male ranged from 0.75 to 3 days with an average of 1.7 ± 0.55 (Table 14). The length and width of adult male mealybug ranged from 1.11mm to 1.23 mm with an average of 1.1 ± 0.03 mm and 0.23 mm to 0.24 mm with an average of 00.23 ± 0.003 mm (Table 15).

Table 15. Morphometrics of life stages of *Phenacoccus solenopsis*

Sl. No.	Stage	Mean* length (mm)	Range (mm)	Mean* width (mm)	Range (mm)
1	Egg	0.37 ± 0.007	0.35 – 0.38	0.18 ± 0.004	0.17 ± 0.19
2	First instar	0.42 ± 0.003	0.415 - 0.426	0.215 ± 0.001	0.212 - 0.218
3	Second instar	0.74 ± 0.06	0.63 - 0.85	0.38 ± 0.03	0.32 - 0.43
4	Third instar	1.42 ± 0.25	1.13 - 1.83	0.71 ± 0.15	0.57 - 1.01
5	Male pupa	1.87 ± 0.02	1.83 – 1.91	0.59 ± 0.01	0.58 - 0.62
6	Adult male	1.1 ±0.03	1.1 - 1.23	0.23 ± 0.003	0.23 - 0.24
7	Adult female	3.5 ± 0.20	3.12 - 3.7	1.92 ± 0.08	1.8 - 2.02

* Mean of 20 observations

4.4 Evaluation of entomopathogenic fungi, botanicals and chemical insecticides under pot culture experiment

Efficacy of entomopathogenic fungi, botanicals and chemical insecticides was evaluated under pot culture experiment. The entomopathogenic fungi at 10^7, 10^8 and 10^9 spores ml^{-1} were applied two times at seven days of interval. Botanicals *viz.*, neem oil emulsion and NSKE (Neem Seed Kernel Extract) were also applied two times at seven days of interval, whereas chemical insecticides *viz.*, buprofezin@ 250 g a.i. ha^{-1} and thiamethoxam @ 25 g a.i. ha^{-1}applied in a single spray (Table 16). Mealybugs were released on one month old okra plants (variety: Arka Anamika) prior to the application of treatments. After 10 days of proper establishment, pre-count of mealybug population was taken. The precount of each treatment was found to be significantly different. At this stage of full fledged mealybug population in the field though varying in nature, first spray of specified treatment was done and subsequent observation was recorded. The pre- treatment count was found to be significant (Table 16). Thus the statistical control of error was maintained and observation was continued. ANOCOVA was done for the 7DAS (7^{th} Day after Spraying) using precount as a covariate.

The results (Table 16) after single day of spraying indicate significant difference in the applied treatments with thiamethoxam 25 g a.i. ha^{-1} (16%), followed by NSKE (13%) and neem oil emulsion (10%). *Lecanicillium lecanii*@ 1×10^8 spores ml^{-1} (8.8%) was on par with *Paecilomyces lilacinus* 1×10^9 spores ml^{-1} (8.6%). After three days of spraying it was found that thiamethoxam 25 g a.i. ha^{-1} caused 40 per cent mortality of mealybug population and the efficacy of buprofezin 250 g a.i. ha^{-1} was increased (25%) than botanicals *viz.*, NSKE (23%) and neem oil emulsion (17%). After five days of spraying the per cent mortality was increased by 63.05 % with the treatment thiamethoxam 25 g a.i. ha^{-1}, which was followed by buprofezin (33.12%) and NSKE (24%). Thiamethoxam 25 g a.i. ha^{-1} showed a

population reduction of 75.94 per cent after seventh day of spraying, which was followed by buprofezin 250 g a.i. ha^{-1} with a population reduction of 37.02 per cent and NSKE 5 per cent with a population reduction of26.52 percent. Treatments with neem oil soap @ 2% (23.09%) was statistically on par with *P. lilacinus* @ 1×10^{7}spores ml^{-1}(20.72%) and *P. lilacinus* @ 1×10^{9} spores ml^{-1}(18.78%). *L. lecanii* @ 1×10^{7} spores ml^{-1}(13.03%) was also statistically on par with *L. lecanii* @ 1×10^{8} spores ml^{-1}(21.89%) followed by *P. lilacinus* @ 1×10^{8} spores ml^{-1}(14%) and *L. lecanii* @ 1×10^{9} spores ml^{-1}(12.02%).Among the entomopathogenic fungi, maximum mortality of *P. solenopsis* was obtained with *P. lilacinus* 1×10^{7} spores ml^{-1}(18.78%)and *L. lecanii* 1×10^{8} spores ml^{-1}(21.89%).

A second spray was resorted at the same level so as to investigate further effect of agrochemicals excluding Thiamethoxam 25 WG and Buprofezin 25 % EC. ANOCOVA was further continued after single day of second spraying by taking precount as a covariate. This process was continued till seventh day of second spray application. Results after 14th day of spraying from first spray application showed (Table 16) maximum reduction of treatments with NSKE @ 5% having 83.54 per cent mortality followed by Thiamethoxam 25 WG with a population reduction of 81.74 per cent mortality. Neem oil soap @ 2 % showed 71.42 per cent mortality which was followed by Buprofezin 25% EC with a mortality of 53.72 per cent.*P. lilacinus* @ 1×10^{9} spores ml^{-1}(45.85%), which was on par with *L. lecanii* @ 1×10^{8} spores ml^{-1}(49.83%). *P. lilacinus* @ 1×10^{8} spores ml^{-1}(32.71%) was on par with *L. lecanii* @ 1×10^{9} spores ml^{-1}(30.87%). It was found that efficacy of all entomopathogenic fungi and botanicals got increased with time lapse after treatment. Maximum reduction of mealybug population was obtained in treatments with chemical insecticides, which showed the residual toxicity of chemical insecticide was more compared to botanicals and microbial pesticides. *P. lilacinus* 1×10^{9} spores ml^{-1} and *L. lecanii* 1×10^{8} spores ml^{-1} were found as the best concentration levels of entomopathogenic fungi applied.

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Table 16. Effect of different agrochemicals on *Phenacoccus solenopsis* in pot culture experiment after first spray

Treatments	Number of mealybug per plant										
	1DBS	1DAS	3DAS	5DAS	7DAS	* % reduction	9 DAS	11 DAS	13 DAS	15 DAS	*% reduction
T_1	111.00	101.4 (83.49)^{cd}	98.08 (80.78)^{bc}	95.66 (77.38)^{bc}	88.08 (70.95)^{cd}	20.72	81.75 (62.69)^{cd}	77.08 (58.83)^{bcd}	74.66 (57.82)^{bcd}	70.41 (54.62)^{b}	36.57
T_2	101.83	93.66 (84.50)^{c}	91.00 (82.04)^{bc}	88.83 (79.35)^{bcd}	87.00 (78.12)^{bc}	14.53	80.50 (65.48)^{bcd}	75.83 (61.45)^{bc}	71.66 (58.40)^{bc}	68.58 (56.14)^{b}	32.71
T_3	102.58	93.66 (83.67)^{cd}	88.7 (79.10)^{bc}	85.91 (75.71)^{b}	83.25 (73.70)^{bcd}	18.78	72.33 (62.29)^{d}	64.41 (54.80)^{d}	59.16 (50.29)^{d}	55.58 (47.26)^{c}	45.85
T_4	89.00	82.75 (85.56)^{bc}	79.00 (81.71)^{bc}	78.50 (81.37)^{bcd}	77.41 (80.10)^{b}	13.03	72.58 (67.52)^{b}	68.83 (63.98)^{b}	64.41 (59.94)^{b}	61.58 (57.39)^{b}	30.89
T_5	89.00	81.41 (85.17)^{bc}	75.33 (78.95)^{bc}	71.50 (75.33)^{b}	69.75 (73.34)^{bcd}	21.89	58.41 (62.04)^{d}	52.58 (56.05)^{cd}	47.25 (50.45)^{cd}	44.83 (47.83)^{c}	49.83
T_6	92.33	89.58 (89.25)^{b}	86.08 (85.76)^{b}	82.58 (82.24)^{cd}	81.16 (80.85)^{b}	12.02	72.91 (63.97)^{bcd}	69.25 (60.69)^{bcd}	65.91 (58.01)^{bcd}	63.83 (56.42)^{b}	30.87
T_7	84.00	75.57 (82.94)^{cd}	69.00 (76.18)^{cd}	66.33 (73.93)^{cd}	64.66 (71.78)^{bcd}	23.09	38.91 (55.61)^{e}	30.41 (46.40)^{e}	25.83 (40.58)^{e}	24.03 (37.92)^{d}	71.42

T_8	91.75	79.50 (79.72)^{de}	70.08 (70.29)^{de}	69.41 (69.64)^{d}	67.41 (67.62)^{d}	26.52	30.25 (51.08)^{f}	20.08 (40.02)^{f}	17.58 (35.99)^{f}	15.16 (32.42)^{d}	83.54
T_9	88.66	*78.08 (81.21)^{cde}	*66.41 (69.43)^{e}	*59.25 (62.44)^{e}	*55.16 (58.15)^{e}	37.02	*47.25 (66.56)^{bcd}	*44.00 (62.49)^{bc}	*42.83 (59.89)^{bc}	*41.00 (57.00)^{b}	53.72
T_{10}	90.41	*75.50 (76.97)^{e}	*54.16 (55.59)^{f}	*33.41 (34.92)^{f}	*21.75 (23.16)^{f}	75.94	*19.33 (67.32)^{bc}	*18.16 (64.11)^{b}	*16.66 (59.07)^{b}	*16.50 (56.26)^{b}	81.74
T_{11}	72.16	78.83 (97.51)^{a}	81.9 (99.94)^{a}	91.33 (110.40)^{a}	103.58 (121.43)^{a}	-	75.48 (75.48)^{a}	134.83 (86.62)^{a}	138.50 (94.00)^{a}	144.41 (102.69)^{a}	-
CD(0.05)		4.15	6.38	6.26	8.13	-	4.25	5.93	5.91	6.26	-

Values in columns are mean of three replications

Figures in parenthesis are adjusted means of number of mealybugs based on ANOCOVA

*Single spray treatment

* Per cent reduction over control

T_1- *Paecilomyces lilacinus* @ 1×10^7spores ml^{-1} T_7-Neem oil soap @ 2 %

T_2-*Paecilomyces lilacinus* @ 1×10^8 spores ml^{-1} T_8-NSKE @ 5%

T_3-*Paecilomyces lilacinus* @ 1×10^9 spores ml^{-1} T_9 - Buprofezin 25 % EC @ 250 ai ha^{-1}.

T_4-*Lecanicillium lecanii* @ 1×10^7 spores ml^{-1} T_{10}-Thiamethoxam 25 WG @ 25 g ha^{-1}

T_5-*Lecanicillium lecanii* @ 1×10^8 spores ml^{-1} T_{11}-Untreated control

T_6-*Lecanicillium lecanii* @ 1×10^9 spores ml^{-1}

4.5 Molecular characterization of gut endosymbionts of mealybug

4.5.1 Isolation and quality checking of gut metagenomic DNA from *Phenacoccus solenopsis*

Metagenomic DNA was isolated from gut of mealybug and presence of 16S rRNA was confirmed with the universal primer 16S rDNA through amplification of the isolated product. An intact and clear band was obtained at 1500 bp when resolved at 0.8 per cent agarose gel (Plate 14). The quality and quantity of metagenomic DNA was observed by using JENWAY Genova Nano Nanodrop, DNA quantified was 2061 ng μl^{-1} with quality 1.91 (A260/A280) indicating good quality of metagenomic DNA. The hypervariable V3 region of 16S rRNA was amplified with specific primer and precede for 16S rRNA library preparation.

4.5.2 16S rRNA library preparation and sample loading

Metagenomic DNA (5 ng) was taken and standard protocol was followed for 16S rRNA library preparation and sample loaded to the Illumina MiSeq™ sequencer.

4.5.3 Illumina sequencing data

Total raw sequencing reads (paired end) of 682,772 with average sequence length of 151 bp was obtained from Illumina MiSeq sequencer. The quality parameters like base quality score distributions (Fig. 1), average base content per head and GC distribution in the reads were checked. It showed that nearly 90 per cent of the total reads had Phred score greater than 30 (>Q30; error-probability>= 0.001).

The base composition distribution of samples were adenine (22.28%), cytosine (25.52%), guanine (29.07%) and thiamine (23.13%). It was observed that the average GC content of each sequence reads ranged from 40-60%. Multiple filters such as region filter, spacer filter and mismatch filter were applied to take high

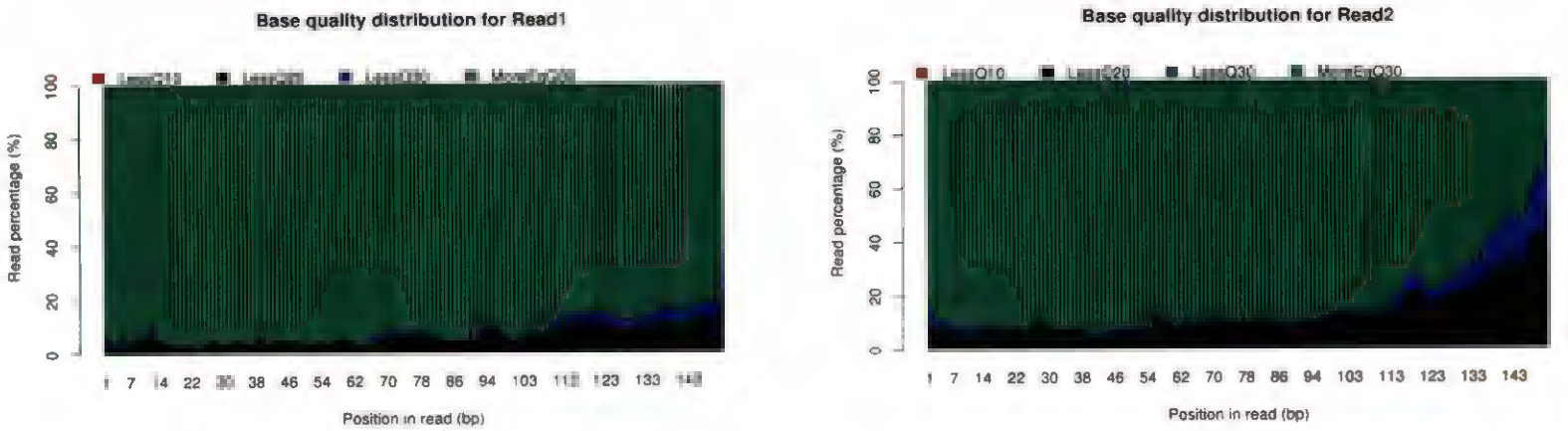


Fig. 1. Base quality distributions of paired end sequences (read 1 and read 2)

M – 100 bp plus DNA ladder, PS – *Phenacoccus solenopsis*

Plate 14. Amplification patterns using universal barcode primers

quality V3 regions for the further downstream analysis, which resulted in 625491, 625006 and 288857 reads respectively. While making consensus V3 sequence, more than 40% of the paired-end reads aligned to each other with zero mismatches with an average contig length of ~135 to ~165bp (Fig. 2).

From the 288,857 consensus reads, singletons and chimeric sequences were removed and thus obtained 272,993 high quality pre-processed reads. The pre-processed reads from sample was pooled and clustered into Operational Taxonomic Units (OTUs) based on their sequence similarity (similarity cut-off 0.97) and a total of 1762 OTUs were identified from 272,993 reads (Fig. 3). Rarefaction analysis was carried out to verify the amount of sequencing reflected in the diversity of original microbial community and the analysis revealed that the species count increased sharply before attaining a plateau (Fig. 4).

4.5.4 Composition of gut bacterial community of mealybug, *Phenacoccus solenopsis*

Composition of bacteria present in the gut of mealybug, *P. solenopsis* was analyzed and grouped them into each taxonomic category from phyla to species level. The abundance of 10 major bacterial groups in each taxonomic category is given in table. We detected altogether 15 bacterial phyla in our sample. Among the phyla, *Proteobacteria* was the most dominant which consisted of 95.93 per cent of total bacterial community, followed by some unclassified bacteria 2.65 per cent (Table 17). Bacteria belongs to *Firmicutes* consist of (1.14%) which was followed by *Bacteroidetes* (0.15%) and *Actinobacteria* (0.05%). Bacteria belongs to *Chlorobi* consist of (0.04%) followed by Streptophyta (0.01%). Bacteria belong to phyla, *Gemmatimonadetes, Planctomycetes, Nitrospirae, Deinococcus* and *spirochaetes* were also recorded in the sample (Fig. 5).

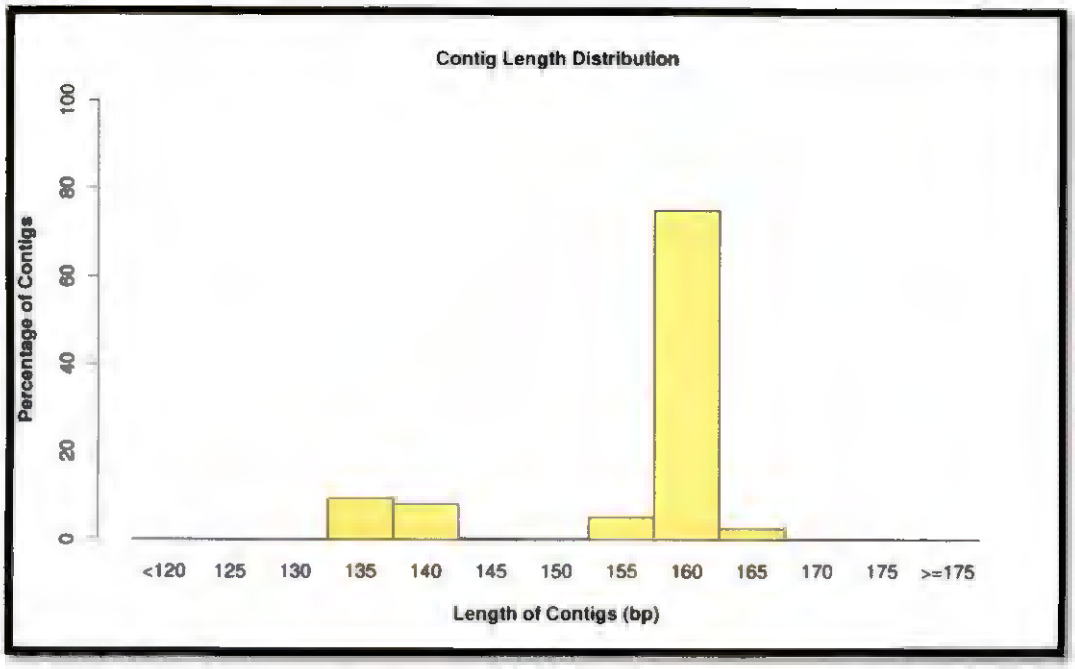


Fig. 2. Contig Length distribution of V3 sequences versus percentage of contigs

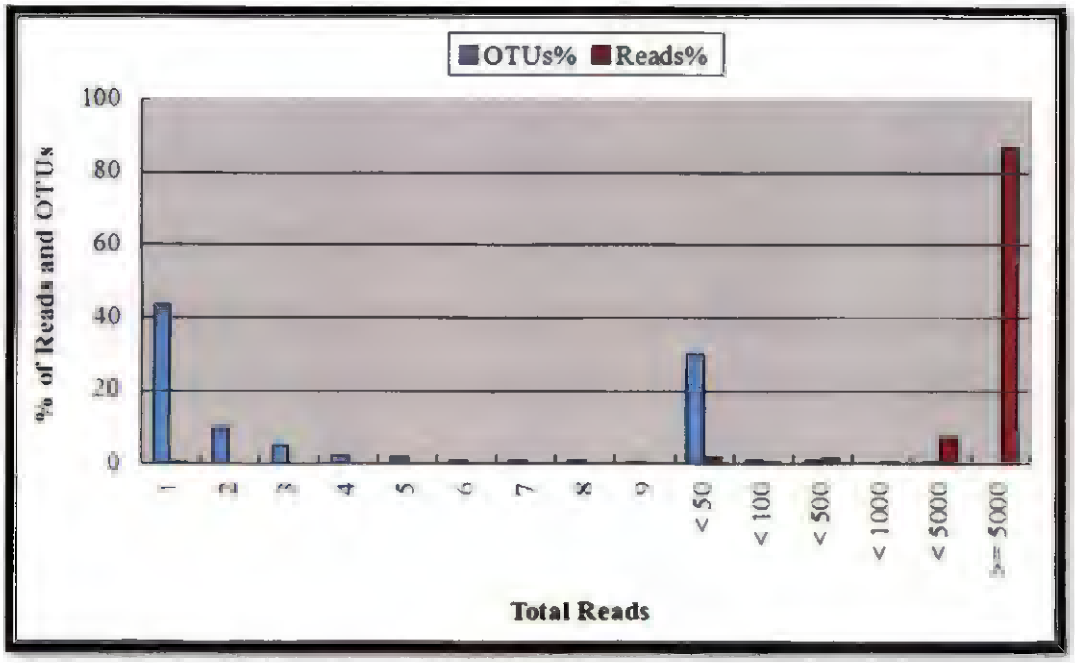


Fig. 3. The percentage of total OTUs and percentage of total read contributed by OTUs

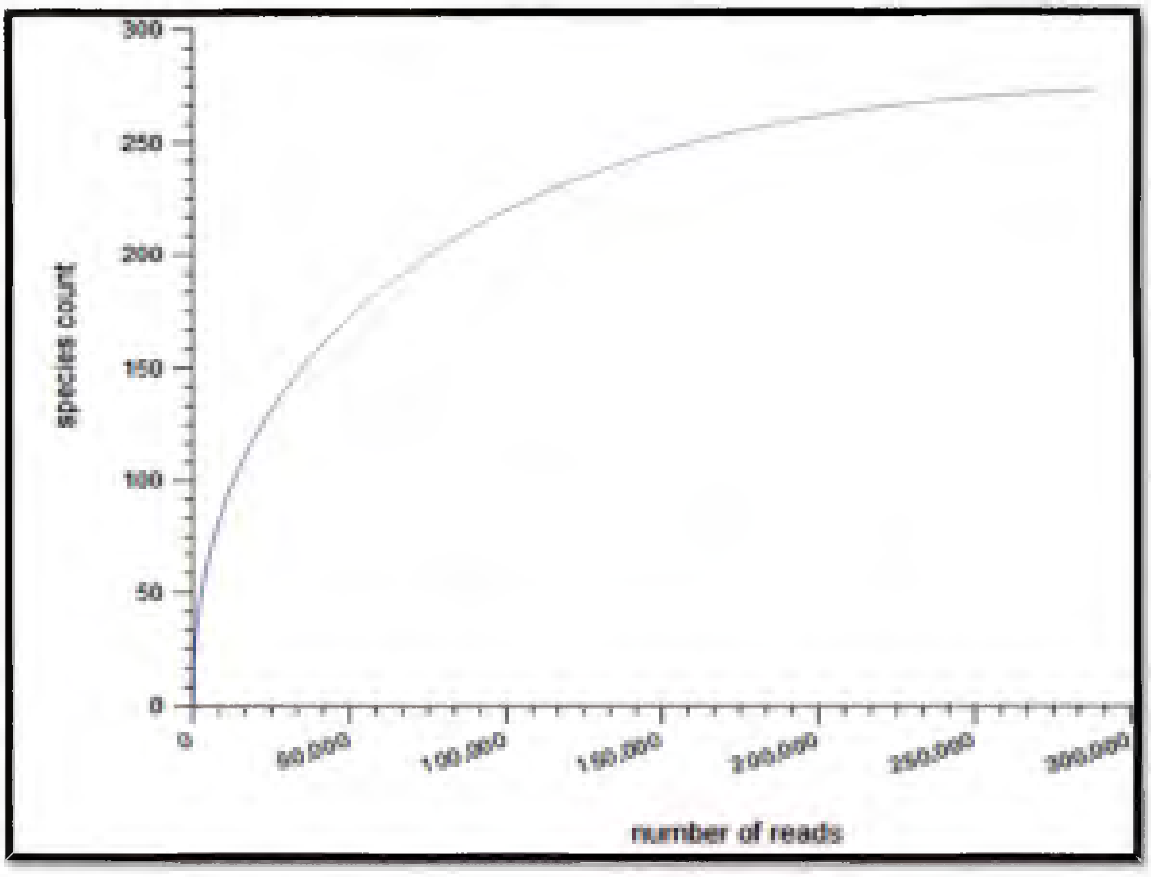


Fig. 4. Rarefaction analyses of *Phenacoccus solenopsis* gut bacterial communities

Total 25 bacterial classes were recorded and identified from the sample of *P. solenopsis*. Among them the most dominant group was *Betaproteobacteria* (71.19%) followed by *Gammaproteobacteria* (24.69%), unclassified (derived from bacteria) (2.65%), *Bacilli* (1.11%), *Bacteroidia* (0.12%), *Actinobacteria* (0.05%), *Chlorobia* (0.05%), *Deltproteobacteria* (0.04%), *Negativicutes* (0.03%), *Flavobacteria* (0.02%), *Sphingobacteria* (0.01%), *Alphaproteobacteria* (0.01%) and *Coniferopsida* (0.01%) (Fig. 6). Later we analyzed the order level which showed altogether, 40 bacterial orders. The most dominant group was unclassified bacteria (derived from *Betaproteobacteria*) (71.15%) followed by *Enterobacteriales* (24.45%), unclassified (derived from bacteria) (2.65%), *Bacillales* (1.08%), *Burkholderiales* (0.16%), *Bacteroidiales* (0.12%), *Vibrionales* (0.06%), *Actinomycetales* (0.05%), *Chlorobiales* (0.04%), *Selenomonadales* (0.03%), *Pseudomonadales* (0.03%), *Lactobacillales* (0.02%) and *Desulfurellales* (0.02%)(Table 17) (Fig. 7).

A total of 63 bacterial families were identified in the sample and unclassified (derived from *Betaproteobacteria*) bacteria (71.19%) was the most dominant among them. Which was followed by *Enterobacteriaceae* (24.45%), unclassified bacteria (derived from bacteria) (2.45%), *Burkholderiaceae* (0.14%), *Bacterodiaceae* (0.11%), *Staphylococcaceae* (0.09%), *Listeriaceae* (0.09%), *Bacilliaceae* (0.09%), *Vibrionaceae* (0.06%), Chlorobiaceae (0.04%), Veillonellaceae (0.03%), *Pseudomonadaceae* (0.03%) and *Desulfurellaceae* (0.02%)(Table 17) (Fig. 8).

Analysis at genus level showed 97 genera in the sample. Among them the most dominant was the *Candidatus Tremblaya* (71.03%), followed by *Klebsiella* (12.27%), *Pantoea* (8.30%), unclassified bacteria (derived from Bacteria) (2.65%), *Wigglesworthia* (1.75%), *Kluyvera* (1.55%), *Staphylococcus* (0.09%), *Enterobacter* (0.43%), *Burkholderia* (0.13%), *Bacteroides* (0.11%), *Listeria* (0.09%), *Bacillus* (0.08%) and *Vibrio* (0.05%) (Fig. 9) (Table 17). A total of 189 species were identified from the sample. *Candidatus Tremblaya princeps* was found as the most dominant

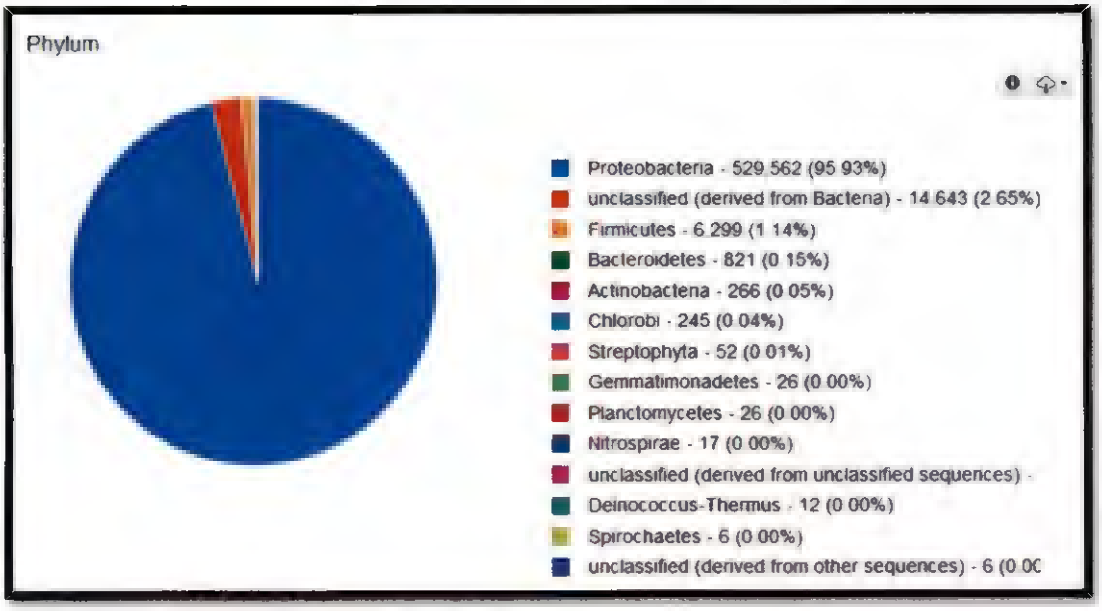


Fig. 5. Abundance of gut bacterial community at phylum level

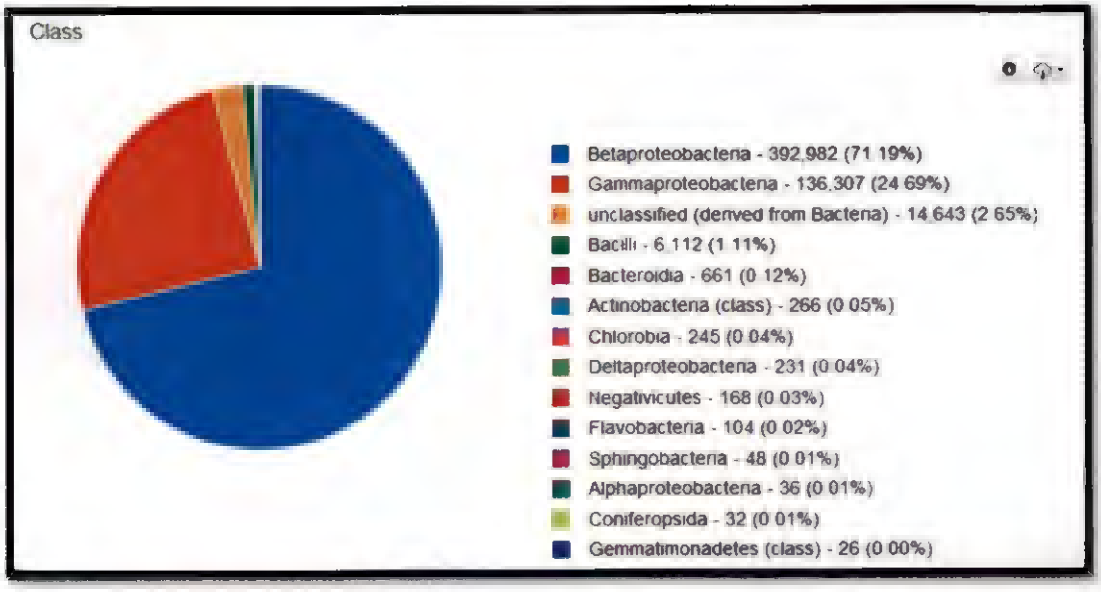


Fig. 6. Abundance of gut bacterial community at class level

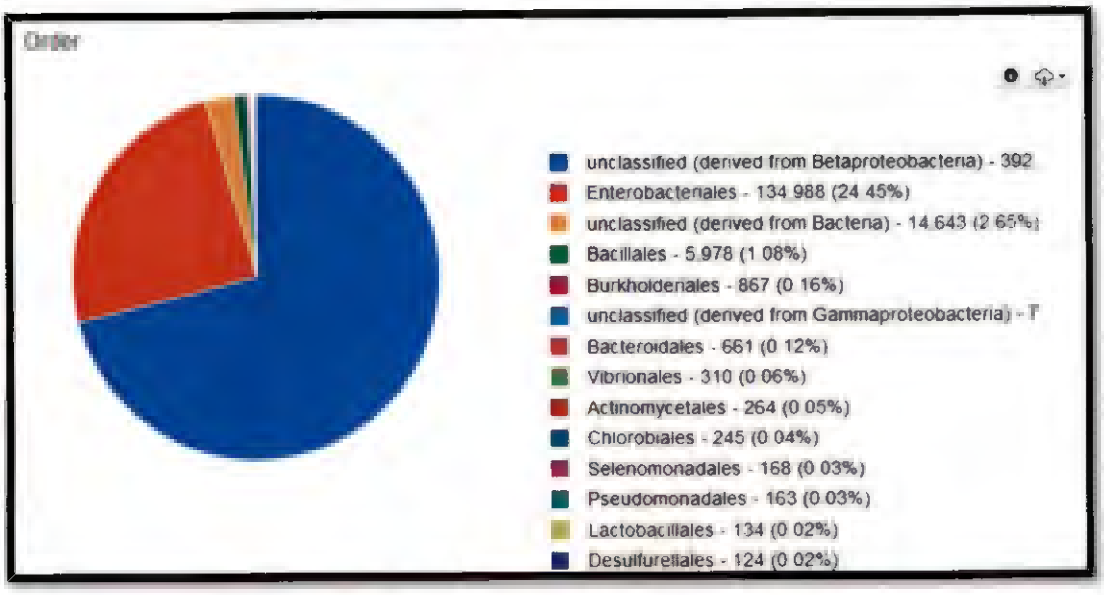


Fig. 7. Abundance of gut bacterial community at order level

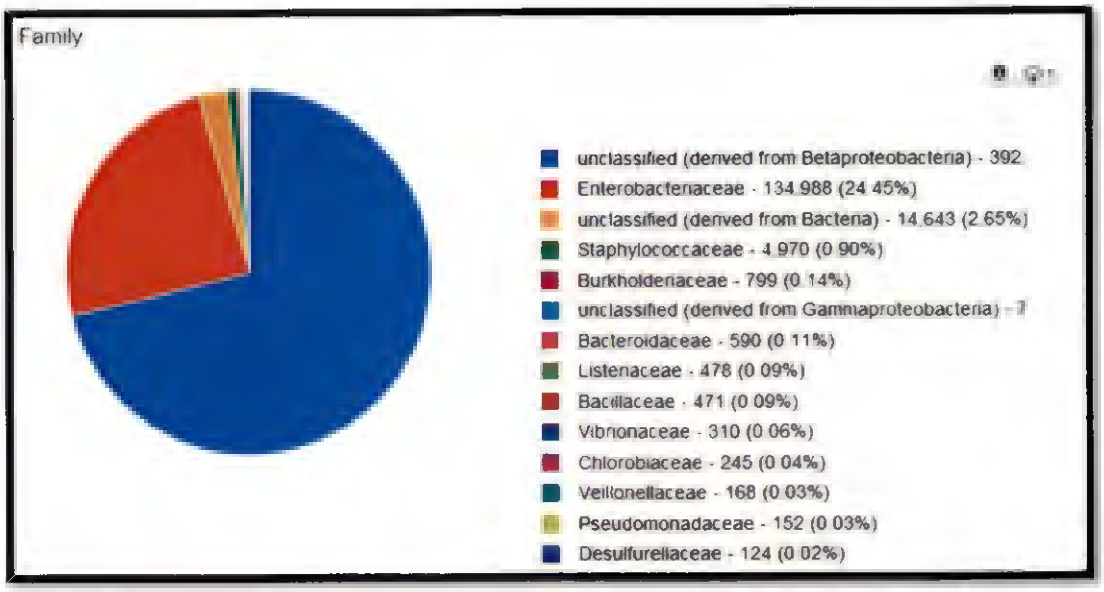


Fig. 8. Abundance of gut bacterial community at family level

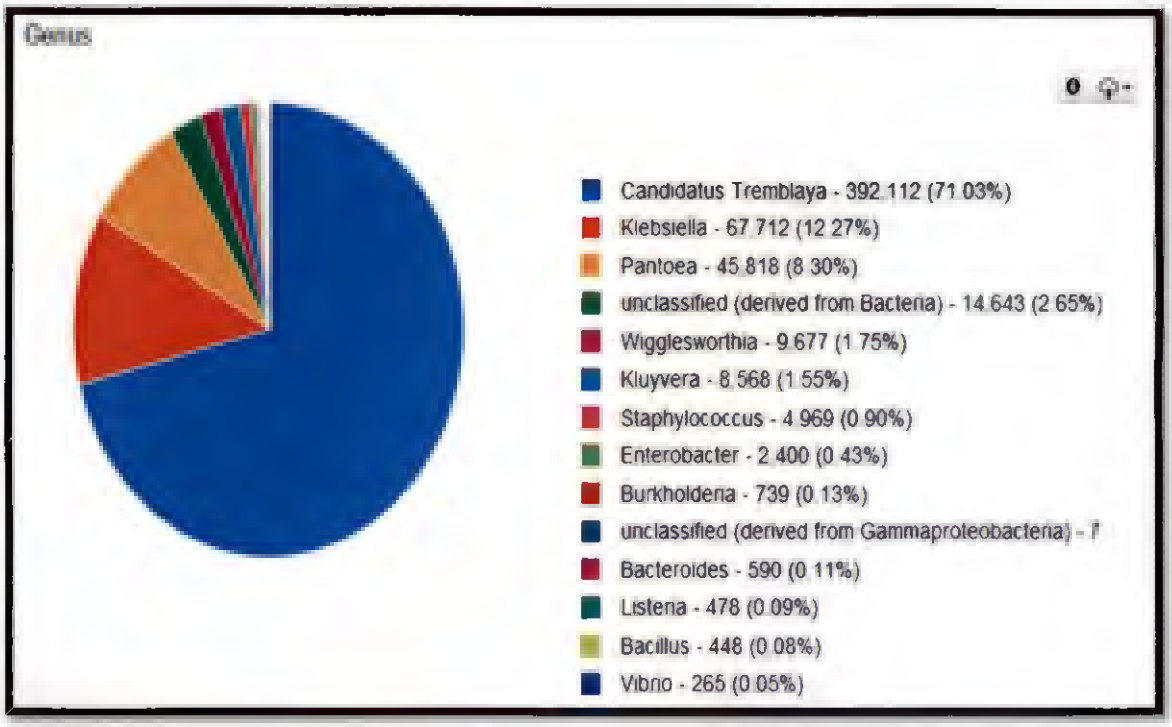


Fig. 9. Abundance of gut bacterial community at generic level

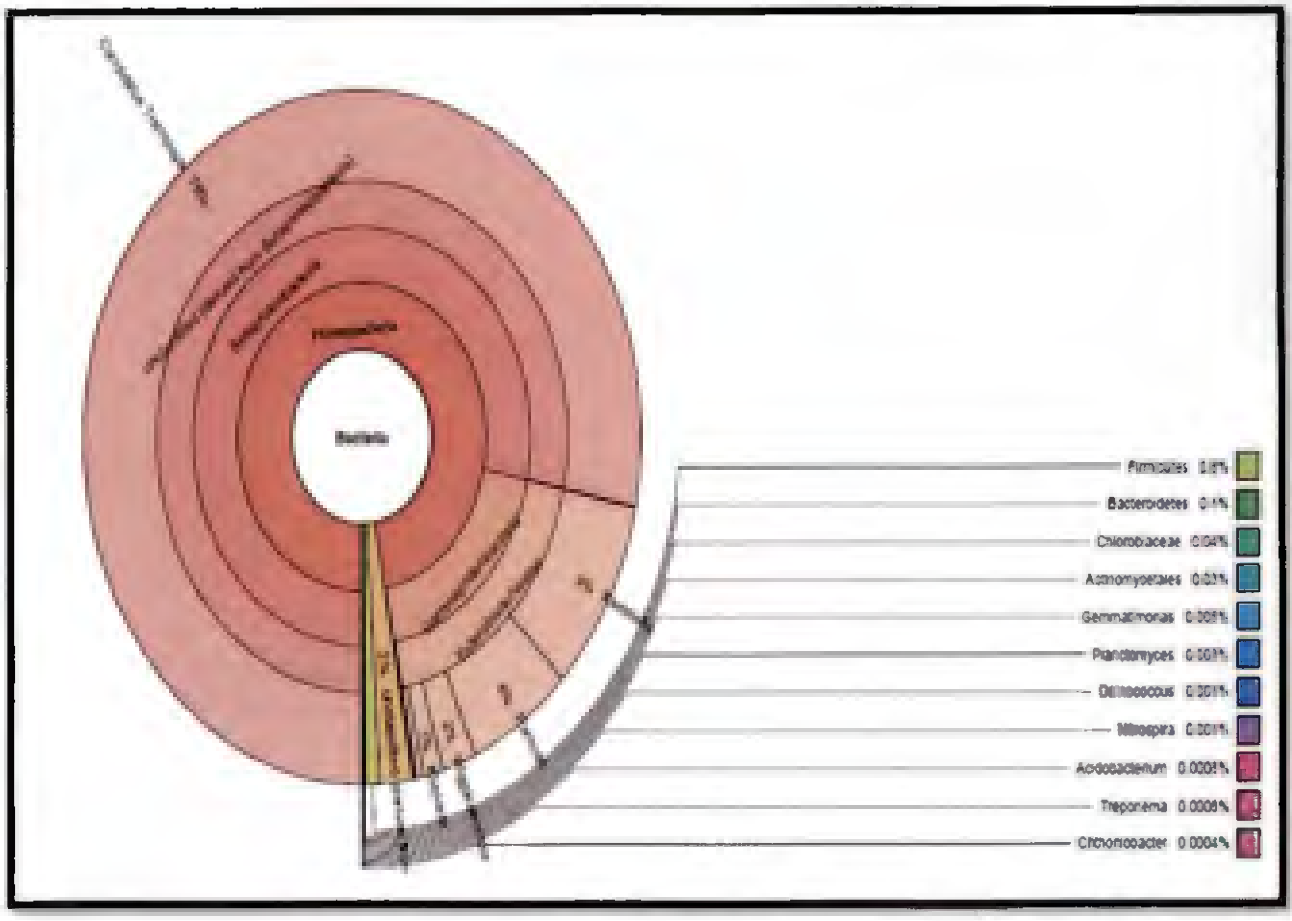


Fig. 10. Abundance of gut bacterial community at species level

(78.28%), followed by uncultured *Klebsiella* sp. (8.83%), *Pantoea agglomerans* (5.98%), *Wigglesworthia glossinidia* (1.93%), uncultured bacterium (1.87%), *Kluyvera ascorbata* (1.14%), *Staphylococcus sciuri* (0.59%), *Enterobacter aerogenes* (0.25%), *Klebsiella pneumoniae* (0.20%),*Pantoea dispersa* (0.13%) and *Listeria grayi* (0.06%) (Fig. 10) (Table 17).

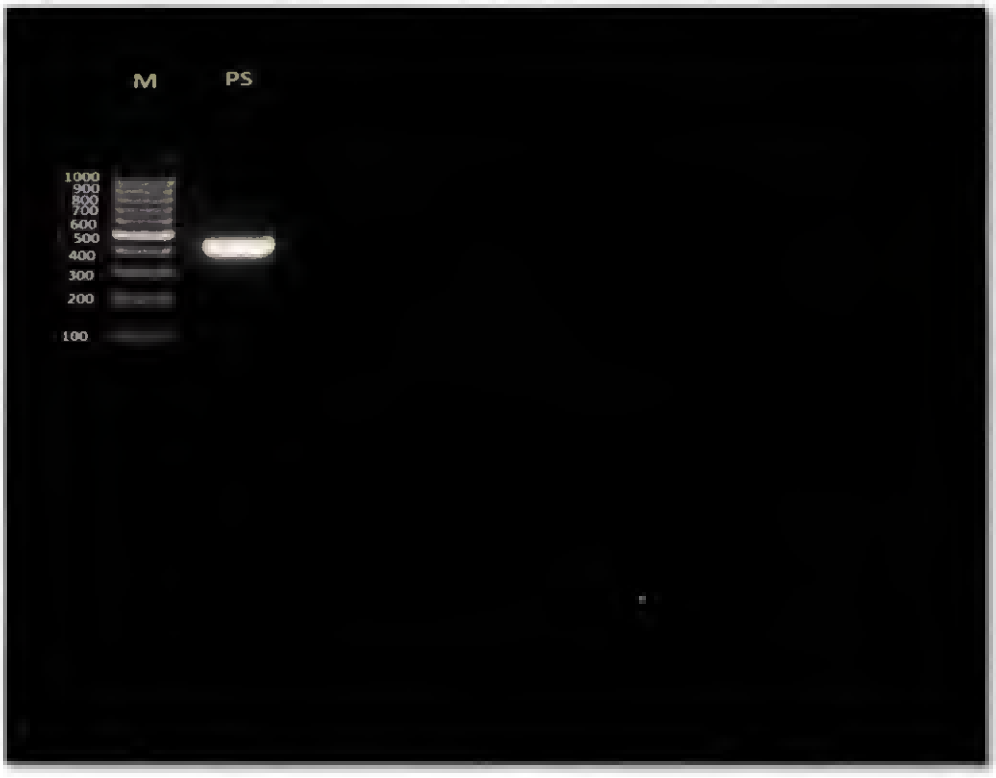
4.5.5Molecular characterization of *Phenacoccus solenopsis*

Genomic DNA of *P. solenopsis* collected from okra plants was isolated through modified CTAB method and intact and clear band was obtained when resolved at 0.8 per cent agarose gel. The quality and quantity of genomic DNA was observed through JENWAY Genova Nano Nanodrop, DNA quantified was 89 ng μl^{-1} with a quality of 1.89 (A260/A280) indicating good quality of genomic DNA.

4.5.5.1DNA barcoding of *Phenacoccus solenopsis*

The mtCOI region was amplified using universal DNA barcode primer in Applied Biosystems Veriti Thermal Cycler and the PCR product gave an intact and clear band at 450 bp when resolved at 1.2 per cent agarose gel (Plate 15). The mtCOI sequence generated from *P. solenopsis* consisted of421 bp and sequence showed significant homology to *P. solenopsis* mitochondrial cytochrome oxidase COI gene already deposited in public domain database using ' blast n' search tool. The blast results showed 100 per cent query coverage and 92 per cent identity to *P. solenopsis* gene. The sequence was aligned and annotated using bioinformatics tools, BioEdit and MEGA6.The sequences thus obtained were submitted to BankIt, NCBIunder accession number, MF770708.

An account was opened in workbench session of BOLD systems v3 database and a new project 'BPSK' was created. Specimen data *viz.*, specimen identifiers, time of collection, specimen details, place of collection *etc.* was submitted and an autogenerated project ID 'BPSK001-17' was obtained. Later, specimen images,



M – 100 bp DNA ladder, PS – *Phenacoccus solenopsis*

Plate 15. Amplification pattern using 16S rDNA primer

primer details, mitochondrial DNA sequences and the trace files obtained from sequencer were uploaded to the database and corresponding barcode of *P. solenopsis* was generated (Plate 16). A phylogenetic tree was constructed using available accessions using Clustal Omega (Plate 17).

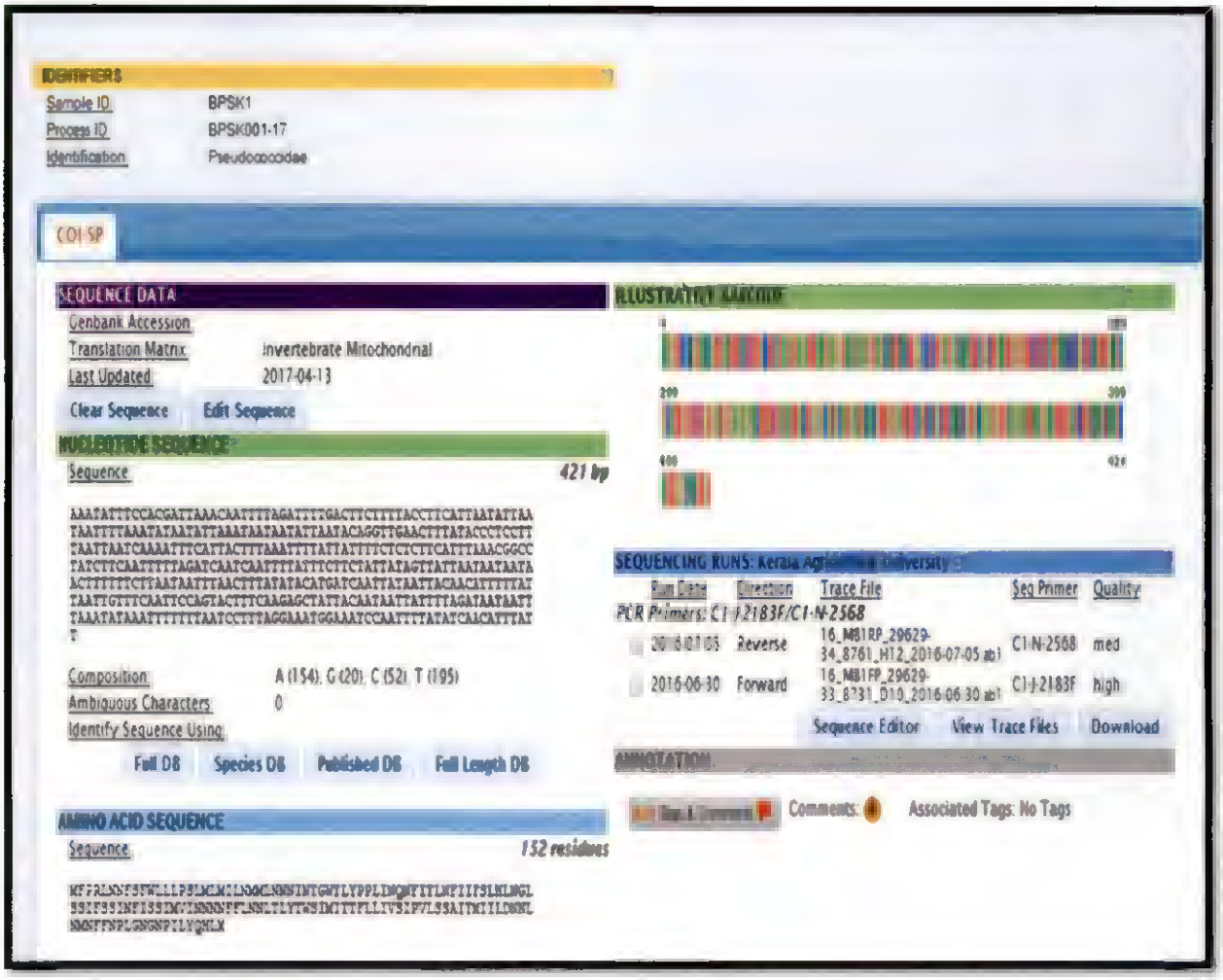


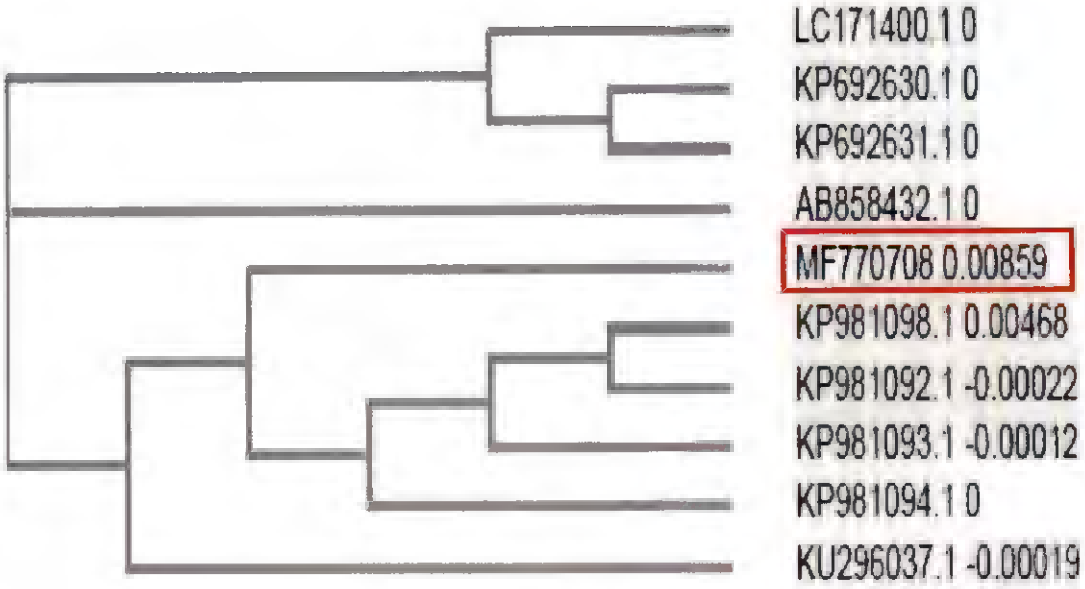
Plate 16. DNA barcode of *Phenacoccus solenopsis* generated by BOLD systems

Table 17. Abundance of major 10 bacterial species from phyla to species occur in the gut of *Phenacoccus solenopsis*

Sl. No.	Phylum	Class	Order	Family	Genus	Species
1	*Proteobacteria*	*Betaproteobacteria*	*unclassified (derived from* Betaproteobacteria)	*unclassified (derived from Betaproteobacteria)*	*Candidatus Tremblaya* (71.03)	*Candidatus Tremblaya princeps* (78.28)
2	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Klebsiella* (12.27)	*uncultured Klebsiella sp.* (8.83)
3	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Pantoea* (8.30)	*Pantoea agglomerans* (5.98)
4	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Wigglesworthia* (1.75)	*Wigglesworthia glossinidia* (1.93)
5	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Kluyvera* (1.15)	*Kluyvera ascorbata* (1.14)
6	*Firmicutes*	*Bacilli*	*Bacillales*	*Staphylococcaceae*	*Staphylococcus* (0.09)	*Staphylococcus sciuri* (.59)
7	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Enterobacter* (0.43)	*Enterobacter aerogenes* (0.25)
8	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Klebsiella* (0.20)	*Klebsiella pneumonia* (0.20)
9	*Proteobacteria*	*Gammaproteobacteria*	*Enterobacteriales*	*Enterobacteriaceae*	*Pantoea* (.13)	*Pantoea dispersa* (0.13)
10	*Firmicutes*	*Bacilli*	*Bacillales*	*Listeriaceae*	*Listeria* (0.09)	*Listeria grayi* (0.06)

Proportion [%] of each category is given in paranthesis

Plate 17. Phylogenetic tree



LC171400.1 : *P. solenopsis* mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds
KP692630.1 : *P. solenopsis* isolate S3-197 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial
KP692631.1 : *P. solenopsis* isolate S3-201 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial
AB858432.1 : *P. solenopsis* mitochondrial COI gene for cytochrome c oxidase subunit I, partial cds
MF770708.0 : *P. solenopsis* mitochondrial COI gene
KP981098.1 : *P. solenopsis* isolate wfsys029 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
KP981092.1 : *P. solenopsis* isolate wfsys023 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
KP981093.1 : *P. solenopsis* isolate wfsys024 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
KP981094.1 : *P. solenopsis* isolate wfsys025 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial
KU296037.1 : *P. solenopsis* voucher NBAIR/MB/PHS/06 cytochrome c oxidase subunit 1 (COX1) gene, partial cds; mitochondrial

DISCUSSION

5. DISCUSSION

Results of work entitled "Population dynamics, biology and management of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera : Pseudococcidae) on okra" with the objectives to study the population dynamics, biology and management of *Phenacoccus solenopsis* Tinsley and characterization of its endosymbionts conducted at AINPAO, College of Horticulture, Kerala Agricultural University, Vellanikkara are summarized in this chapter.

5.1 Survey and documentation of cotton mealybug, *Phenacoccus solenopsis* and their associated fauna

Survey was conducted in Thrissur district from April 2016 to May 2017, where different places under five selected blocks of Thrissur were visited in monthly intervals. Okra growing areas were thoroughly observed for assessing the damage caused by the mealybug as well as recording the natural enemy fauna associated with the mealybug. Unfortunately, the population of mealybug was low during surveys. Instead, several alternate host plants were noticed with mealybug infestation and natural enemies of mealybug were also recorded from there itself. About 45 host plants were noticed with infestation of mealybug, *P. solenopsis* and which included, vegetable crops *viz.*, chilli, brinjal, amaranth, tomato and several other plants such as China rose, flannel weed, wire weed, Siam weed, Levant cotton, long stalk sida, goat weed *etc.* The host plants were confined to families *viz.,* Asteraceae, Malvaceae, Solanaceae, Cucurbitaceae, Amaranthaceae *etc.* (Fig. 11). Arif *et al.* (2009) also documented about 154 host plants of *P. solenopsis* which included 20 field and horticultural crops, 45 ornamentals, 64 weeds and 24 bushes and trees. Deshmukh *et al.* (2009) recorded about 91 host plants of mealybug distributed under 24 families. It shows that cotton mealybug is highly invasive and polyphagous. Mealybug thrives on alternate host plants like weeds and ornamentals during off seasons and cause serious damage to crop plants at their early stages itself.

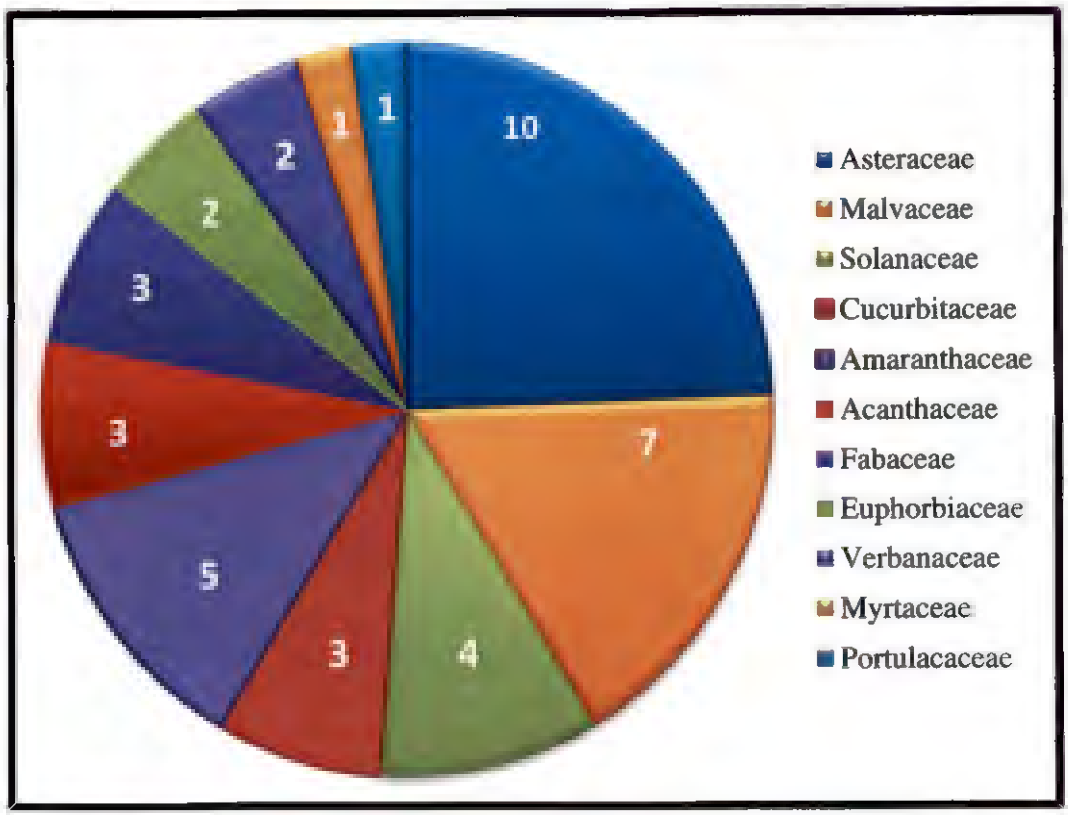


Fig. 11. Host range of *Phenacoccus solenopsis*

Natural enemies were recorded, one among them was predator of mealybug, *Spalgis epius* (Westwood) and the others were parasitoid, *Aenasius arizonensis* (Girault), *Anicetus* sp., *Myiocnema comperei* Ashmead and *Prochiloneurus* spp. Arve *et al.* (2011) also reported the presence of *S. epius* predating mealybug, *P. solenopsis*. Arif *et al.* (2012), reported predators *viz., Brumoides suturalis* (Fabricius), *Scymnus coccivora* Ayyar, *Menochilus sexmaculata* (Fabricius), *Coccinella undecimpunctata* (Linnaeus), *Hyperaspis maindroni* Sicard, *Chrysoperla carnea* (Stephens) and *Geocoris* sp. feeding on the cotton mealybug. The predator was found throughout the year, but the population of predator decreased during monsoon season compared to summer and post – monsoon season. Similarly population of parasitoids also reduced during monsoon season. *A. arizonensis* was the most common predator of *P. solenopsis*, while *M. comperei* and *Anicetus* sp. was rarely observed. Tanwar *et al.* (2008) reported large scale parasitism of *A. bambawalei* with *P. solenopsis* from four different places *viz.*, Hisar, New Delhi and Parbhani. During the survey they also recorded another parasitoid, *Promuscidea unfasciativentris* Girault which turn the mealybug into legless, brown, barrel shaped mummy. The parasitoids could be released on weeds and ornamentals which act as the alternate host of *P. solenopsis*, prior to cultivation of crop plants. Studies of Nagrare *et al.* (2009) suggested that release of ladybug beetle, *Cryptolaemus montrouzieri* Mulsant would be beneficial in suppressing population of *P. solenopsis* when released upon weeds and other perennial plants prior to cotton cultivation. During the surveys, *S. acuta*, *A. indicum* and *H. rosa- sinensis* were bearing mealybug population throughout the year and parasitoid, *A. bambawalei* was also observed along with the population of mealybug. Amutha *et al.* (2009), showed the natural parasitization of *P. solenopsis* by *Aenasius* sp. among several host plants, among them the maximum parasitization was found to be on *A. indicum* (5 to 65%) followed by *Parthenium hysterophorous* (5 to 30%). On the contrary, Zhou *et al.* (2015) reported that the *P. solenopsis* experienced less per cent of parasitism when it was on *Parthenium hysterophorous* when compared to the

other host plants like, *H. rosa- sinensis* and *G. hirsutum*. In the present study, maximum parasitization of *P. solenopsis* was found to be with *A. arizonensis*. Similarly, Muniappan (2009) had reported 60 per cent parasitization of *P. solenopsis* by *A. bambawalei* at field level. Vijaya *et al.* (2010) observed the extent of parasitization by *A. bambawalei* on *P. solenopsis* which ranged from 28.65 to 58.97 per cent from the month of April to October, reaching its peak during November. In the present study, the population of *A. arizonensis* was more in the month of March to July and which found decreased during the monsoon season and again increased during the post monsoon season. Maruthadurai and Singh (2015) reported 6.31 to 53. 52 per cent parasitization of mealybug by encyrtid parasitoid, *A. arizonensis*. In present study, diseased mealybugs were absent, but pathogens infecting *P. solenopsis viz., Aspergillus clavatus, A. oryzae, A. terreus* and *Lecanicillium lecanii* were reported by Gulsarbanu *et al.* (2009).

5.2 Assessment of damage caused by *Phenacoccus solenopsis*

Assessment of damage caused by *P. solenopsis* was done by visual scoring of leaf (Galanihe *et al.,* 2011) and the severity of damage was calculated. Damage assessment studies on okra plant in polyhouse revealed that there were little crinkling produced by first instar nymphs on okra leaves when they were released with 2 to 8 number of nymphs per plant. Both one week old and one month old okra plants produced crinkling symptoms when they were infested with 10 number of first instar mealybugs. It showed that, even though feeding of first instar nymphs caused less crinkling, the per cent crinkling would be increased along with an increase in number of mealybugs. Second instar and third instar mealybugs produced crinkling symptoms when they were released with a pest load of 6 to 10 number of mealybugs per plant on both one week old and one month old okra plants. Adult mealybugs released with a pest load of 8 to 10 number per plant could only produce crinkling symptoms on one week old okra plants. Adult mealybugs produced no crinkling symptoms on one month old okra plants. The results resembled with that of the

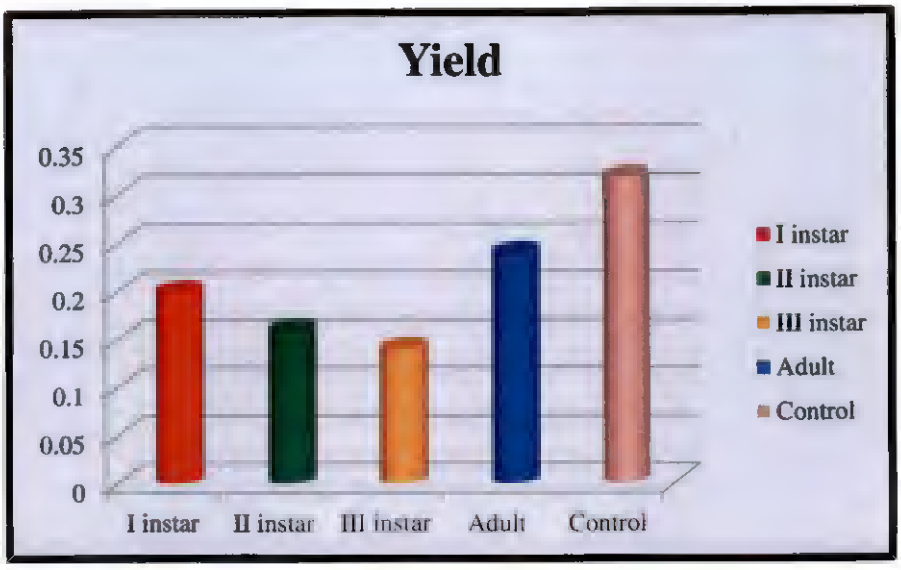
crinkling caused by papaya mealybug, *Paracoccus marginatus* Williams & Granara de Willink on papaya as reported by Galanihe *et al.* (2011), where they found that adult mealybugs produced very less crinkling on leaves. Bhosle *et al.* (2009), found that the mealybug, *P. solenopsis* was mainly present on young growth of plant including stems, leaves and twigs. *P. solenopsis* was mainly present on the young growth including twigs, leaves, flower buds and petioles (Hodgson *et al.*, 2008), which was similar with the present study. Mealybug attack caused loss of quantity as well as quality of crop produce. Significant reduction in yield was observed due to the feeding of second and third instar mealybugs than the first instar and adult stages (Fig. 12).

5.3 Biology of *Phenacoccus solenopsis*

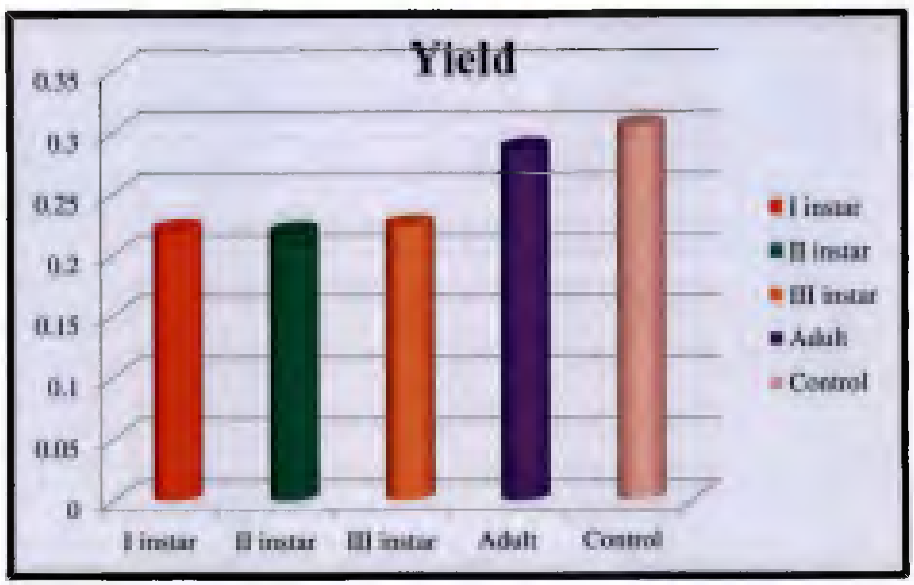
Laboratory studies on biology of *P. solenopsis* was carried out on okra twigs. There were three nymphal instars and an adult stage in female mealybug (Fig. 13), while adult male possessed two nymphal instars with a pre-pupa and a pupal instar (Fig 14). Crawlers were probably produced by ovo-viviparous and parthenogenetic mode of reproduction.

5.3.1 Life stages of *Phenacoccus solenopsis*

The life cycle of *P. solenopsis* comprised of three nymphal instars in female and two nymphal instars in male. Male mealybug possessed an extra pre-pupal and pupal period prior to attain the adult stage. Morphometric differences were observed in each instar of mealybug. The total nymphal period in *P. solenopsis* ranged from 12.5 to 18.5 days with an average of 15.3 ± 1.74 days in female and 16.5 to 23.75 days with an average of 18.88 ± 1.62 days in male. This might be due to the additional pupal instar in male mealybugs. Vennila *et al.* (2010) also observed that the average nymphal duration of male mealybug was slightly longer as compared to female mealybug. They recorded that the male mealybug took an average of 18.9 ± 0.9 days of nymphal period while female mealybug completed their nymphal period



Yield variation of one week old plants caused by mealybug infestation



Yield variation of one week old plants caused by mealybug infestation

Fig. 12. Yield variations on one week old and one month old okra plants due to mealybug infestation

in an average of 13.2 ± 1.8 days. Badshah *et al.* (2015) also recorded that the average of total nymphal period of *P. solenopsis* on okra was 18.8 ± 0.4 days.

5.3.2 First instar nymph of *Phenacoccus solenopsis*

The newly emerged first instar nymphs were pale yellowish in colour, dorsally convex and were having six segmented antenna. Freshly emerged first instar nymphs were crawled over to the leaf surface and settled down for feeding. Hodgson *et al.* (2008) also confirmed the presence of six segmented antenna in first instar nymph of *P. solenopsis*. The nymphal period ranged from 3 to 6.5 days with an average of 4.27 ± 0.84 days. The result of Vennila *et al.* (2010) was in conformity with the present result as their observations on nymphal duration of first instar was 3.9 ± 0.4 days. Jat (2011) also observed that the nymphal duration of first instar nymph was 4.3 ± 1.02 days. Observations of Kedar *et al.* (2010), was also similar to the present study, where they reported the first instar nymphal duration was 4.72 ± 0.68 days.

Morphometric observations of *P. solenopsis* showed that the first instar nymphs were having a mean length of 0.42 ±.003 mm and a mean width of 0.215 ± 0.001 mm. Huang *et al.* (2012) also reported that the mean length of *P. solenopsis* measured was 0.62 ± 0.02 mm and their mean width ranged from 0.22 ± 0.02 mm, which was in accordance with the present observations.

5.3.3 Second instar nymph

Second instar nymphs were observed to be oblong in shape and were also in yellowish colour with six antennal segments. Even though antenna was six segmented, they showed slight difference in size of segments. There was presence of two pairs of blackish spots over the dorsal side of their body. The second instar nymphs were started secreting waxy powder after 24 to 46 hours from first moult. Muthulingam and Vinobaba (2013) also observed the same mode of behavior in

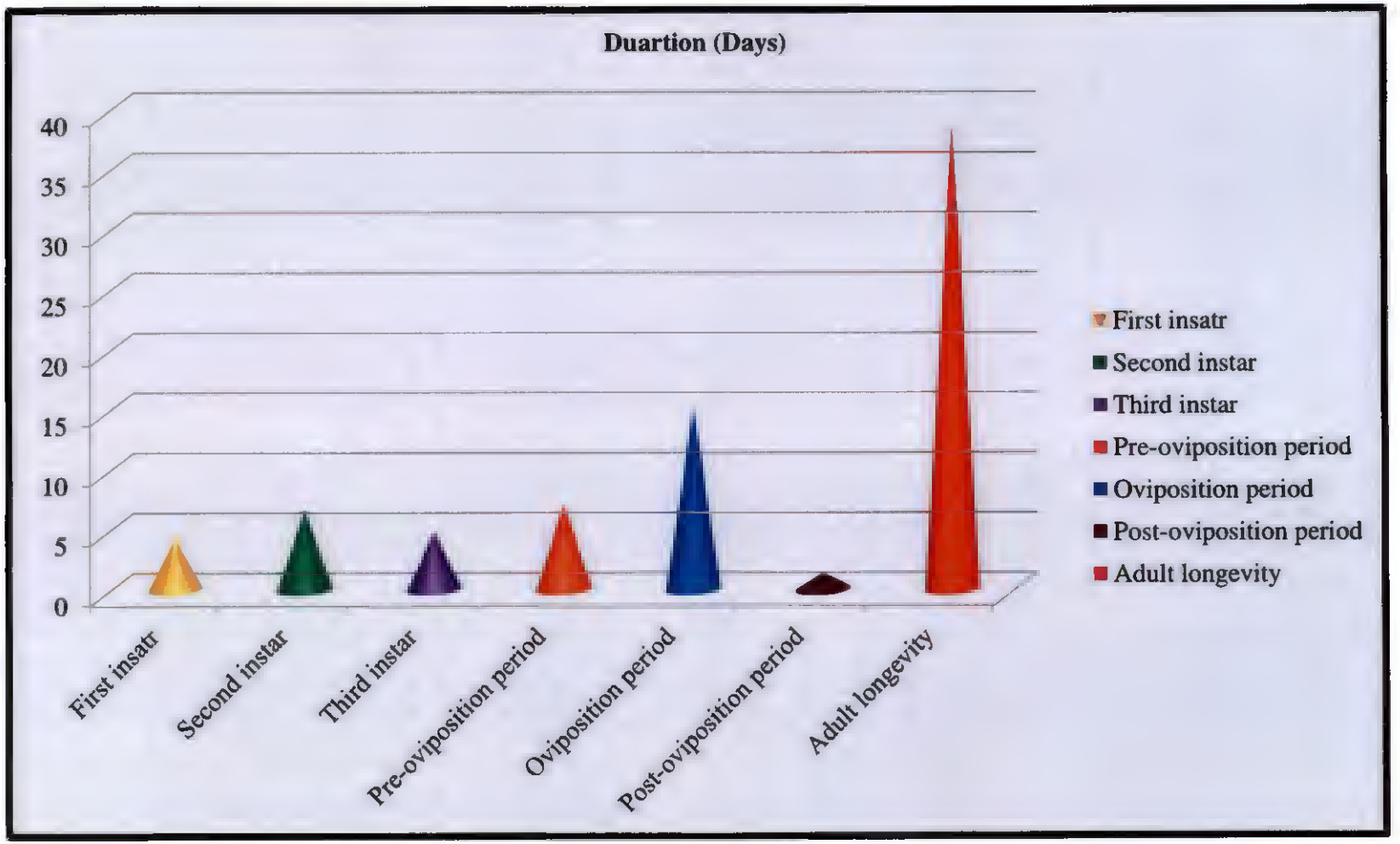


Fig. 13. Duration of developmental stages of *Phenacoccus solenopsis* (Female)

second instar nymphs of *P. solenopsis* that, there was secretion of white waxy powder and waxy fibres on dorsal side of body after 24 hours of first moult. According to Hodgson *et al.* (2008) antennal segments observed in second instar crawlers were also six in number. Average duration of second instar nymphs were 6.67 ± 0.97 days, which was in conformity with the results obtained by Vennila *et al.* (2010) where they recorded the average nymphal period as 5.1 ± 3.2 days. Kedar *et al.* (2010) also recorded that the nymphal duration of second instar nymphs were 5.2 ± 0.71 days.

The mean length and width of second instar nymph of *P. solenopsis* recorded was 0.74 ± 0.06 mm and 0.38 ± 0.03 mm, whereas, Huang *et al.* (2012) recoded the mean length of second instar nymphs were 1.13 ± 0.06 mm and mean width was 0.47 ± 0.02 mm, which was slightly longer as compared to the length and width values obtained in present study.

5.3.4 Third instar nymph

Third instar nymphs were fully covered with wax coating over the dorsal side of their body. They were oblong shaped with two dark coloured black spots over the dorsal side of their body. They possessed seven segmented antenna. Hodgson *et al.* (2008) also observed the third instar nymphs of *P. solenopsis* with seven segmented antenna. Muthulingam and Vinobaba (2013) confirmed the presence of two pairs of black spots over the dorsal side of body in third instar nymph. The average duration of third instar in female mealybug was, 4.8 ±1.33 days. Vennila *et al.* (2010) also recorded that the average duration of third instar nymph in P. solenopsis was 4.2 ± 0.6, which was similar with the present study. Kedar *et al.* (2010) observed that the average nymphal duration of third instar nymphs were 5.2 ± 0.71 days which was also similar with the values recorded.

Morphometric observations on third instar nymph of mealybug recorded was, 1.42 ± 0.25 mm and 0.71 ± .15 mm, as the mean length and width. Similar values

were recorded by Huang *et al.* (2012) where they observed the average length of third instar nymphs as 1.68 ± 0.07 mm and the average width as 0.72 ± 0.03 mm.

5.3.5 Pre-pupal period and pupal stage in male mealybug

Male mealybug was found to possess pre-pupal period and a pupal stage prior to adult stage. After the second nymphal instar, the mealybugs were started to produce white silken cocoons. They continued to moult inside the cocoons and remain inside the pupal case itself before attaining the adult stage. Male pupal case was cylindrical in shape. Muthulingam and Vinobaba (2013) also observed similar characteristics of male mealybug where they found that the male mealybug cocoons were cylindrical in shape with white colour. The average duration of pupal period in male was 6.8 ± 1.06 days. Similar observations were made by Vennila, *et al.* (2010) where the mean pupal period was 5.5 ± 0.5 days. The mean length of male pupa ranged from 1.87 ± 0.2 mm and the mean width was 0.59 ± 0.01 mm. The average length and width of pupa was almost similar with the observations of Huang *et al.* (2012) where he recorded 1.58 ± 0.05 mm average length and 0.59 ± 0.03 mm average width of male pupa.

5.3.6 Pre-oviposition period, oviposition period, post- oviposition period and fecundity of *Phenacoccus solenopsis*

In females, the average pre-oviposition period recorded was 7 ± 1.5 days which ranged from 5 to 9.5 days. Oviposition period lasted for 11.5 to 20 days with an average of 15 ± 2.07 days. The post- oviposition period ranged from 0.5 to 2 days with an average of 1.35 ± .58 days. According to Kedar *et al.* (2010) the pre-oviposition, oviposition and post oviposition periods were 5.96 ± 0.73, 10.08 ± 1.12 and 3.00 ± 0.76 days respectively, which was slightly different from the observed values due to varied weather conditions. While, Vennila *et al.* (2010) recorded the pre-oviposition, oviposition and post-oviposition periods which was almost similar, where they recorded 5.7 ± 1.7, 17.2 ± 4.3 and 2.4 ± 0.6 days respectively.

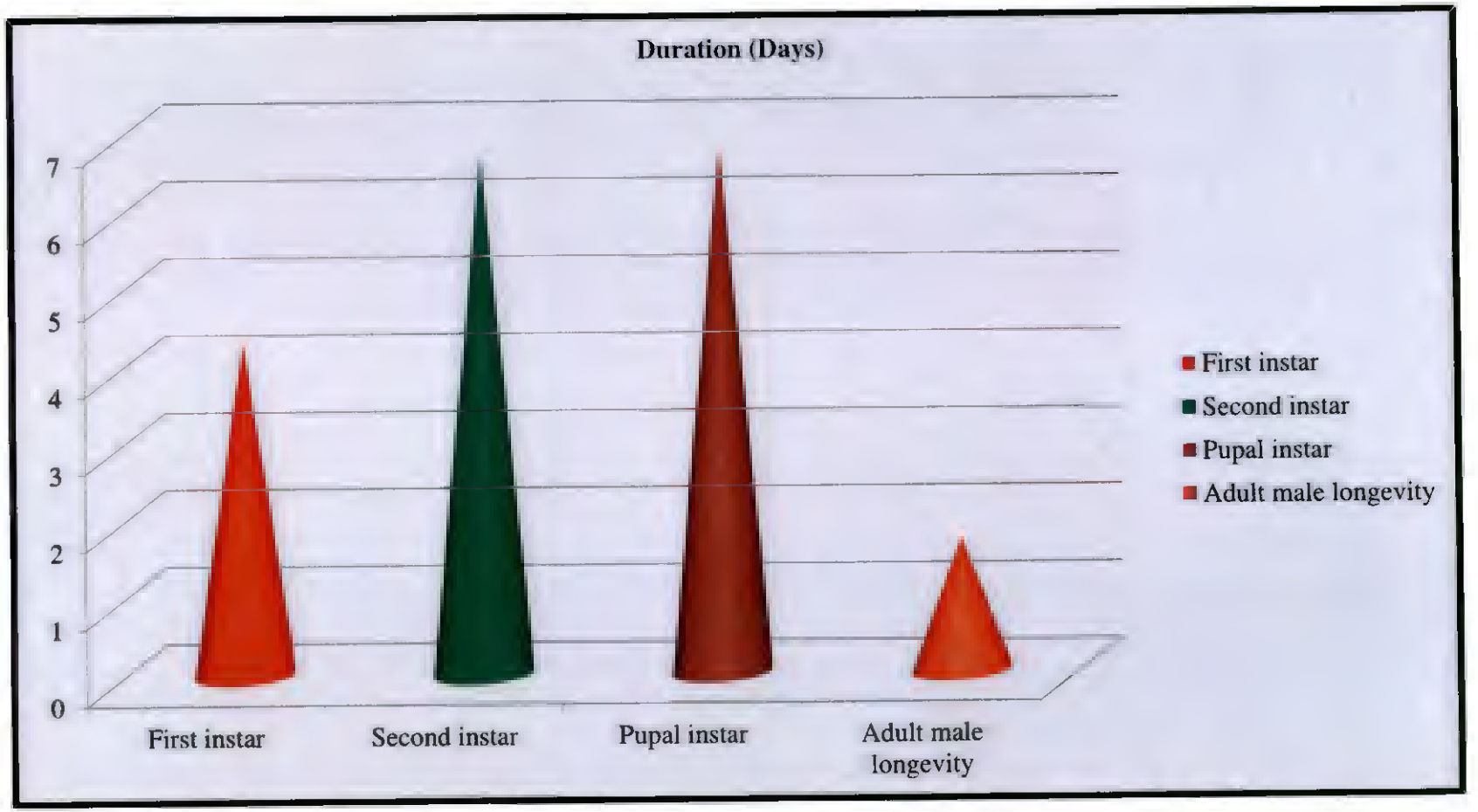


Fig. 14. Duration of developmental stages of *Phenacoccus solenopsis* (Male)

Fecundity of mealybug, *P. solenopsis* was 121 to 218 with an average of 171 ± 28.15. Abbas *et al.* (2009) also observed that the average fecundity of mealybug was about 111.9 when the mealybug was reared upon okra plant. Vennila *et al.* (2010) recorded the fecundity as 158 to 812 when reared upon cotton, which was comparatively more than that of the present study. The difference in fecundity might be due to the host plant characteristics.

5.3.7 Adult

Females and males of *P. solenopsis* differed morphologically. Female mealybugs were apterous, oblong shaped. They possessed 18 pairs of cerrarii. The body of female mealybug was fully covered with thick waxy coating. They were having nine segmented antenna. There were two pairs of black spots/strips over the dorsal side of their body. Male mealybugs were very delicate, elongate and possessed ten segmented antenna. They were having a pair of well developed milky white wings. There were two pairs of waxy filament at the anal end of their body. The inner filaments were longer than the outer filaments. The adult female mealybugs lived for about 34 to 44.5 days with an average of 38.75 ± 2.8 days. Vennila *et al.* (2010) recorded the mean longevity of female mealybug as 42.4 ± 5.7 days which ranged from 36 to 51 days. Studies conducted by Singh and Kumar (2013) got similar observations. They recorded 39.88 ± 3.12 days as the female longevity period. The adult male was short lived and the mean duration of adult was 1.7 ± 0.55 days. Similarly, Vennila *et al.* (2010) observed that the average duration of adult male was 1.5 ± 0.1 days. Singh and Kumar (2013) also found similar observations that the adult male duration was 1.96 ± 0.84 days.

The mean length of adult female mealybug was found to be 3.5 ± 0.50 mm and the mean width was 1.92 ± 0.08 mm. Adult male mealybug was having a mean length of 1.1 ± 0.03 mm and a mean width of .23 ± .003 mm. Huang *et al.* (2012) also observed similar morphometric values, as the mean length and width of female

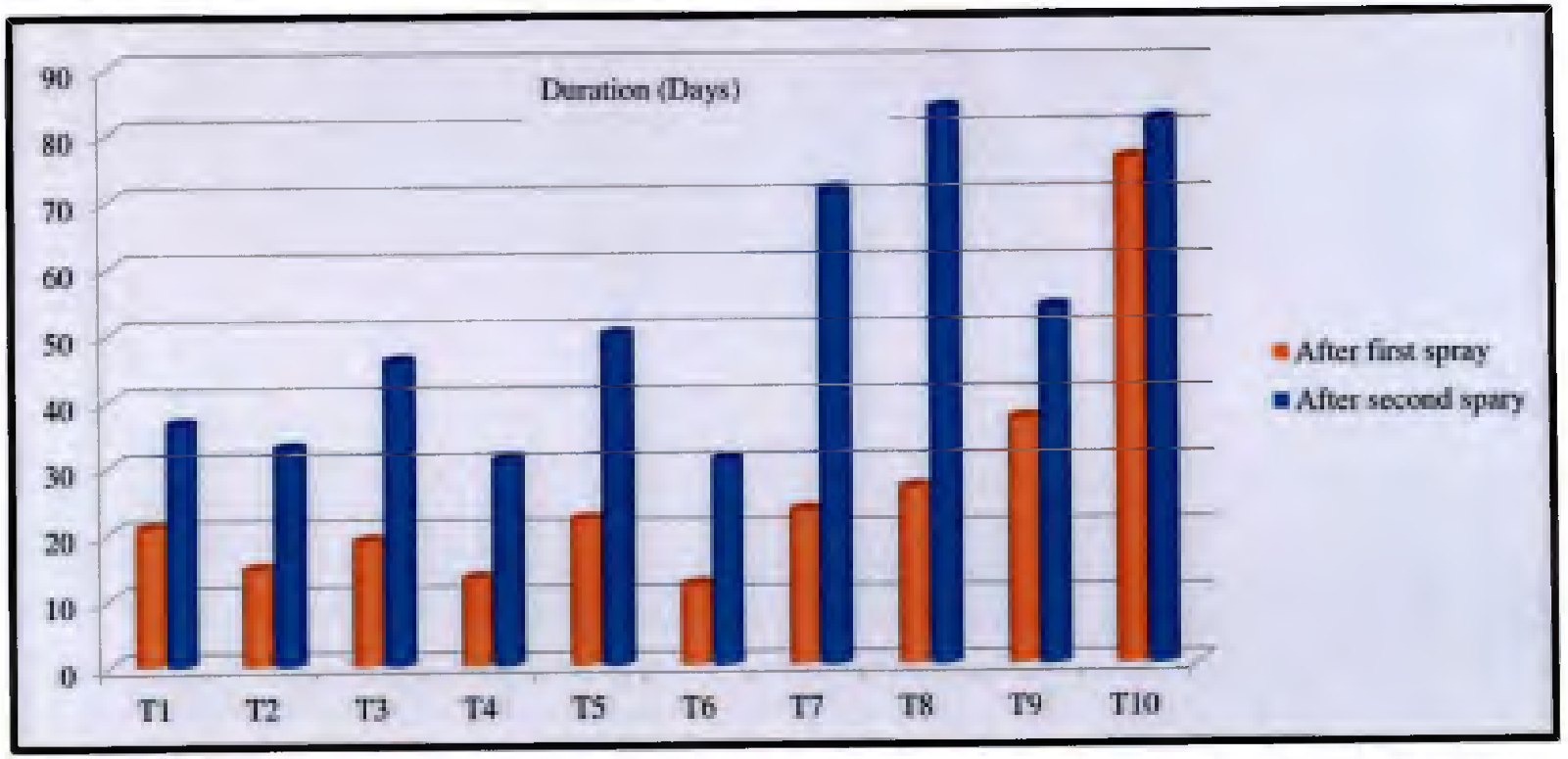
mealybug was 3.12 ± 0.08 mm and 1.60 ± 0.03 mm respectively, while the mean length of adult mealybug was 0.99 ± 0.06 mm and the mean width was 0.19 ± 0.01 mm.

5.4 Evaluation of entomopathogenic fungi, botanicals and chemical insecticides under pot culture experiment

Efficacy of different agrochemicals *viz., Paecilomyces lilacinus* (Thom) Samson, *Lecanicillium lecanii* R. Zare & W. Gams, neem oil emulsion, NSKE, buprofezin 250 g a.i. ha^{-1} and thiamethoxam 250 g a.i. ha^{-1} were evaluated under pot culture experiment. *P. lilacinus* and *L. lecanii* were applied in three concentrations *viz.*, 1×10^7, 1×10^8 and 1×10^9 spores ml^{-1}. Two sprays were resorted to understand the efficacy of different agrochemicals on mealybug population except for thiamethoxam 25 g a.i. ha^{-1} and buprofezin 250 g a.i. ha^{-1}. The observation of number of live mealybugs were counted at 1, 3, 5 and 7 days after each spray.

Single spray of thiamethoxam 25 g a.i. ha^{-1} was found as the most promising treatment after seven days of initial application of treatments (Fig. 15). Maximum mortality of mealybug was observed with the chemical insecticides and botanicals which might be due to their immediate action of interruption of physiology in insects. Rishi *et al.* (2009) also reported the efficacy of chemical insecticides in controlling *P. solenopsis*, where he found that profenofos @ 1250 ml, monocrotophos @ 1250 ml, chlorpyriphos @ 3000 ml, quinalphos @ 2000 ml, acephate @ 2000 g a.i., thiodicarb @ 625 g and carbaryl WP @ 2500 g/ha were very effective against the mealybug. Halder *et al.* (2013) found that *P. solenopsis* showed 70.29 per cent mortality on application with neem oil (5%), while entomopathogenic fungi *viz., B. bassiana*, *Metarhizium anisopliae* Metschn and *L. lecanii* showed 62.85, 56.52 and 67.11 per cent mortality (Fig. 15). *Lecanicillium lecanii* @ 1×10^8 spores ml^{-1} and *Paecilomyces lilacinus* @ 1×10^9 spores ml^{-1} were very effective compared to other concentrations of the fungi *viz., L. lecanii* and *P. lilacinus* @ 1×10^7 spores ml^{-1}, in

Fig. 15. Effectiveness of the entomopathogenic fungi, botanicals and chemical insecticides on *Phenacoccus solenopsis* in pot culture experiment



T1- *P. lilacinus* @ 1×10^{7} spores ml^{-1}, T2- *P. lilacinus* @ 1×10^{8} spores ml^{-1}, T3- *P. lilacinus* @ 1×10^{9} spores ml^{-1}, T4- *L. lecanii* @ 1×10^{7} spores ml^{-1},T5- *L. lecanii* @ 1×10^{8} spores ml^{-1},T6- *L. lecanii* @ 1×10^{9} spores ml^{-1}, T7- Neem oil emulsion @ 2 %, T8- NSKE @ 5% , T9- Buprofezin 25 % EC @ 250 ai ha^{-1},T10- Thiamethoxam 25 WG @ 25 g ha^{-1}, T11- Untreated control

present study. Amutha and Banu (2015) also recorded the effectiveness of *L. lecanii* agaist mealybug, *P. marginatus*. They found that the *L. lecanii* required more penetration time than *M. anisopliae* and *B. bassiana*, so that the time required for mortality of mealybugs were also longer compared to other fungal pathogens. In present study, mortality of mealybugs observed after application of microbial pesticides was effective after 7 DAS which support the findings of Amutha and Banu (2015).

After second spray, maximum mortality was still observed with chemical insecticides and botanicals (Fig. 15). Thiamethoxam 25 WG and buprofezin 25 % EC showed highest mortality of mealybug in okra followed by botanicals, such as neem oil soap (2%) and NSKE. Mamoon-ur-Rashid *et al.* (2011) found that neem oil at 1.5 and 2 per cent concentration caused significant reduction in mealybug population, however found less toxic compared to other chemical insecticides, which was supporting the present study. Surulivelu *et al.* (2012) reported that the treatments with chemical insecticides such as, acephate and chlorpyriphos caused mortality of mealybug, *P. solenopsis* of about, 87.1 to 93.8 per cent compared to other microbial pesticides *viz., B. bassiana, L. lecanii* and *M. anisopliae* which were moderately effective and caused a mortality of 39.1, 30.9 and 28.2 per cent respectively.

5.5 Molecular characterization of endosymbionts of *Phenacoccus solenopsis*

An attempt was made to explore the bacterial communities associated with *P. solenopsis*, as the feeding bahaviour of *P. solenopsis* was influenced by the gut bacterial communities. Isolation of gut metagenomic DNA of *P. solenopsis* was done by SDS based metagenomic DNA extraction procedure explained by Zhou *et al.* (1996). Illumina Next Generation Sequencing platform was used to reveal the total bacterial community present in the gut. Analysis of hypervariable V3 region of 16S rRNA fragment resulted in large bacterial community with 1762 OTUs per sample with 92 per cent identity detection.

The analysis of bacterial community at phyla level revealed that *Proteobacteria* was the most dominant in the gut of *P. solenopsis*, which was followed by some uncultured bacteria, *Firmicutes, Bacteroidetes* and *Actinobacteria*. Similarly, Szabo (2017) reported that most of the pseudococcinae mealybugs harbor a unique symbiosis setup with betaproteobacterial symbionts which is coming under *Proteobacteria*. Parkinson (2016) also reported the nested symbiosis in citrus mealybug, *Planococcus citri* (Risso), in which phylum *Proteobacteria* was the dominant one. Douglas, (2009), reported the dominance of *Proteobacteria* in haemocoel of sap feeding aphids and psyllids, however he found that *Bacteroidetes* was dominant along with *Proteobacteria* in glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar). *Firmicutes* was the dominant phylum in *Glossina fuscipes fuscipes* as reported by Lindh and Lehane (2011). Bacterial communities belonged to *Bacteroidetes* and *Firmicutes* were dominant in the gut of termites (Xiang *et al.*, 2012).

Proteobacteria was associated with diverse metabolic and physiological properties such as, cell envelope biogenesis, synthesis of essential amino acids and B vitamins in insects (Benett *et al.*, 2014). Kikuchi *et al.* (2012) reported that *Proteobacteria* in insects helped in pesticide detoxification. *Proteobacteria* assisted in carbohydrate metabolism of insect body which helped in cellulose degradation in insects (Dalalibera *et al.*, 2005). According to Brown *et al.* (2012) *Firmicutes* in insects were highly beneficial as they helped in cellulose and hemicellulose degradation in insects. Present study revealed the presence of entomopathogens, *Bacillus thuringiensis* and *Bacillus cereus* which were coming under the *Firmicutes* in the gut of *P. solenopsis*. Gruwell *et al.* (2007) found that *Bacteroidetes* in Diaspididae promoted the evolution of paternal genome elimination and thereby increased feminization in insect populations. Schrempf, (2001) reported that *Actinobacteria* helped in production of extracellular enzymes and wide variety of secondary metabolites.

Candidatus Tremblaya princeps was found as the major species of bacteria in the gut of *P. solenopsis*. Parkinson (2016) reported the presence of *Candidatus Tremblaya princeps* in the gut of citrus mealybug, *Planocccus citri* which exhibited nested endosymbiosis with another symbiont namely, *Candidatus Moranella endobia*. *Pantoea agglomerans* was another bacterium associated with the gut of *P.solenopsis*. Dillon and Charnley (1995) reported the presence of *Pantoea agglomerans* (Ewing and Fife) in desret locust, *Schistocerca gregaria* (Forsskal) which helped in production of phenol that inhibited conidia germination of *Metarhizium anisopliae*. *Wigglesworthia glossinidia* was also found in gut of *P. solenopsis*, which were having genes encoding for synthesizing B vitamins *viz.*, pantothenate (Vitamin B_5), biotin (Vitamin B_7), thiamin (Vitamin B_1), riboflavin (Vitamin B_2), pyridoxine (Vitamin B_6), nicotinamide (Vitamin B_3) and folate (Vitamin B^9) in its genome. (Akman *et al.*, 2002). *Acidobacterium capsulatum* was present in the gut of *P. solenopsis*. *Acidobacterium* sp. was also reported by Reid *et al.* (2011) as xylanolytic bacteria in termite gut. Interestingly, gut bacterial community exhibited the presence of bacteria, *Erwinia amylovora*, which is reported to be causing fire blight in apple, pear and other ornamentals (Vanneste, 2000). *Bacillus pumilus* bacteria was found associated within the gut of mealybug, which was reported to be producing high amount of physiologically active gibberellins (Gutierrez- Manero *et al.*, 2001) and cellulose enzyme (Ariffin *et al.*, 2006). *Candidatus Regiella insecticola*, which was also present in gut of mealybug, was reported to be present in pea aphid, *Acyrthosiphon pisum* that helped to prevent the attack from fungal pathogens. Another bacteria detected in mealybug gut was, *Escherichia coli*, which was reported to elicit effective immunity against lethal and highly virulent insect pathogen, *Photorhabdus lumininescens* TT01 in *Manduca sexta* caterpillars (Eleftherianos *et al.*, 2006). *Lactobacillus acidophilous* were present in mealybug gut, which was also reported by Vilela *et al.* (2015) that the presence of *Lactobacillus acidophilous* in *Galleria mellonella* which inhibited the biofilm

formation by *Candida albicans*. *Micrococcus* sp. recorded in sample was also recorded by Bulet *et al.* (1999) as it was involved in synthesis of antimicrobial peptides which acted as defensive compounds against insect pathogens. *Paenibacillus* sp. HanTHS1 was found in the bacterial community of mealybug gut. This was reported by Bouraoui *et al.* (2016) as they isolated a multifunctional enzyme named, GH51 arabinofuranosidase from *Paenibacillus* sp. Surprisingly, the entomopathogenic bacteria, *Photorhabdus temperata* was present in gut of *P. solenopsis*. Jung and Kim (2006) reported that the *Photorhabdus temperata* can be used against *Spodoptera exigua* as a synergist with *B. thuringiensis*. *Raoultella ornithinolytica* were associated with the bacterial community in the analysed sample. Kanki *et al.* (2002) reported that the *Raoultella ornithinolytica* could produce histamine poison which might kill fish through histamine fish poisoning. Another bacteria identified in mealybug sample was *Verticillium dahliae*. Bhat and Subbarao (1999) reported that *Verticillium dahliae* exhibited a very specific host range which included cotton, bell pepper, cabbage, egg plant and cauliflower. *Xenorhabdus* sp. were also present in mealybug sample, which was known to be associated with entomopathogenic nematodes (Jung and Kim, 2006). The uncultured *Burkholderiales* bacterium was found associated with gut bacterial community, which was capable of detoxifying pesticides. Kikuchi (2012) reported that the *Burkholderia* sp. associated with stink bugs utilize organophosphorous compounds as sources of carbon, nitrogen and phosphorous by facilitating detoxification of these compounds.

Future line of work

- Biochemical analysis of alternate host plants of *P. solenopsis* to reveal the reasons behind off season survival.
- Confirmation studies on the effect of entomopathogenic fungi and botanical insecticides through large scale multi-location trials in different seasons.
- Confirmation studies on functional diversity of endosymbionts of *P. solenopsis*.

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Summary

6. SUMMARY

The study entitled " Population dynamics, biology and management of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera : Pseudococcidae) on okra" was conducted at Department of Agricultural Entomology, College of Horticulture, KAU, Vellanikkara during February 2016 to May 2017 and the results of investigation are summarized below.

- Priliminary surveys were conducted to record the incidence of *P. solenopsis* Tinsley in okra growing areas of Thrissur district. Major five blocks of Thrissur district were selected to record the population dynamics of *P. solenopsis* on okra. As there were no infestations of *P. solenopsis* on okra plants during the survey period (March 2016 to March 2017), the population dynamics of okra was decided to be conducted on major weed hosts of mealybug *viz., Sida acuta* Burm. f., *Abutilon indicum* (Link) Sweet, *Hibiscus rosa-sinensis* L. and *Amaranthus viridis* L. Infested host plants were scored based on the scale from zero to four.
- Natural enemies and ants associated with *P. solenopsis* were collected and identified. *Spalgis epius* (Westwood) was the only major predator found associated with the mealybug during the population studies, which was identified from Department of Agricultural Entomolgy, College of Horticulture, Vellanikkara. Parasitoids were identified by Dr, Mohammed Hayat of Aligarh Muslim University *viz.*, *Aenasius arizonensis* (Girault), *Anicetus* sp., *Myiocnema comperei* Ashmead and *Prochiloneurus* spp. The ant species associated with mealybug, *P. solenopsis* was identified as *Anoplolepis gracilipes* (Smith) by Dr. K. A. Karmaly, Department of Zoology, St. Xavier's College, Aluva.

- Damage assessment of one week old and one month old okra plants was done in pot culture experiment by releasing 2, 4, 6, 8 and 10 numbers of mealybugs per plant. Experiment showed that third instar and second instar mealybugs of *P. solenopsis* were causing higher percentage of crinkling in okra leaves as compared to first instar and adult mealybugs.
- *P. solenopsis* population was collected from mealybug infested okra field of Agricultural Research Station, Pattambi during June, 2016 and specimen was sent to NBAIR, Bengaluru for species confrmation. Further mass culturing of *P. solenopsis* population was carried out on potato sprouts and pumpkin fruits at AINPAO, KAU, Vellanikkara. Twenty first instar nymphs of uniform stages were taken from the mass culture of *P. solenopsis* to carry out the biology studies. Biology of mealybug, *P. solenopsis* was carried out on okra twigs of 5 cm length. In the present study, it was found that in female mealybug, ovo-viviparity and parthenogenesis were the major mode of reproduction. Morphometric observations and presence of exuvia during each moult of mealybug helped to distinguish different instars of mealybug.
- The average nymphal duration of male mealybug (18.88 ± 1.6 days) was slightly longer as compared to female mealybug (15.3 ± 1.74 days) due to the presence of an additional pupal period. The first instar crawlers were green-yellowish and with dorso-ventrally flattened body. The duration of first instar crawlers was 3 to 6.5 days and measured an average of 0.42 mm in length and 0.21 mm in width. Second instar crawlers were partially covered with wax and were having yellowish flattened body. The duration of second instar crawlers were found to be 5 to 8.5 days and measured an average length and width of 0.76 mm and 0.38 mm, respectively. Third instar nymphs were yellowish and fully covered with wax over their body. Duration of third instar nymphs ranged from 3 to 8.5 days with an average length of 1.42 mm and width of 0.71 mm. Male mealybug possessed an additional pupal instar which

measured with an average length of 1.87 mm and width of 0.59 mm. The duration of pupal stage ranged from 5.25 to 9 days.

- Adult female mealybugs were apterous and had a convex yellowish body which was covered with thick waxy coating. Adult male mealybugs were winged, delicate and possessed four anal filaments. Outer anal filaments were short as compared to inner filaments. Adult female had longevity of 34 to 44.5 days, whereas adult male had longevity of only 0.75 to 3 days. The pre-oviposition period of female mealybug ranged from 5 to 9.5 days and an oviposition period of 11.5 to 20 days. Adult female had their post-oviposition period of only 0.5 to 2 days. Number of crawlers produced per female ranged from 125 to 218 and the female to male sex ratio was found to be 1: 0.03.
- Effectiveness of entomopathogenic fungi (*Lecanicillium lecanii* (Zimm.) and *Paecilomyces lilacinus* (Thom) Samson), botanicals (NSKE@ 5% and Neem oil soap @ 2%) and chemical insecticides (buprofezin 25% EC and thiamethoxam 25 WG) was evaluated in pot culture experiment on 30 days old okra plants (variety: Arka Anamika). After first spray, thiamethoxam 25 WG showed immediate effect with a population reduction of 75.94 per cent followed by buprofezin 25% EC (37.02%). NSKE caused a per cent mortality of 26.52 per cent. Treatments with neem oil soap and *P. lilacinus* @ 1×10^7 spores ml^{-1} was on par with *P. lilacinus* 1×10^9 spores ml^{-1}. Among the entomopathogenic fungi, *P. lilacinus* @ 1×10^9 spores ml^{-1} (18.78%) and *L. lecanii* 1×10^8 spores ml^{-1} (21.89%) was found as the best treatments. Results after 14^{th} day of first spray (7^{th} day after second spray) showed treatments NSKE @ 5% caused a population reduction of 83.54 per cent which was on par with thiamethoxam 25 WG (81.74%). *P. lilacinus* 1×10^9 spores ml^{-1} caused a percent mortality of 45.85 per cent which was on par with *L. lecanii* 1×10^8 spore ml^{-1} (49.83%). It was found that efficacy of all

entomopathogenic fungi and botanicals got increased with time lapse after treatment.

- Molecular characterization of endosymbionts of *P. solenopsis* was carried out and diversity of gut inhabiting bacteria was analysed based on Illumina Next Generation Sequencing of 16s rRNA amplicons. The data consisted total raw sequencing reads of (paired end) 682, 772 with an average sequence length of 151 base pairs. A total of 1762 OTUs were identified from 272, 993 reads.
- A total of 15 bacterial phyla, 25 classes, 40 orders, 63 families, 97 genera and 189 species were identified from the sequence analysis. *Proteobacteria* was the most dominant group, followed by *Firmicutes* and *Bacteroidetes.*
- The search on function of different gut inhabiting bacteria of *P.solenopsis* revealed their role in nutrition, detoxification of lethal insecticide molecules like organophosphorous and defensive action against pathogens. Insecticidal toxin producing bacterial species were also found in gut of *P. solenopsis.*

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POPULATION DYNAMICS, BIOLOGY AND MANAGEMENT OF MEALYBUG, *Phenacoccus solenopsis* Tinsley (HEMIPTERA: PSEUDOCOCCIDAE) ON OKRA

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ABSTRACT OF THE THESIS

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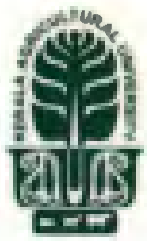

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ABSTRACT

Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a highly polyphagous pest that infest more than 154 species of plants mostly belonging to Asteraceae, Malvaceae, Solanaceae, Amaranthaceae, and Euphorbiaceae. It causes severe damage to crops grown under both protected as well as open field conditions. Use of broad spectrum synthetic insecticides to manage the mealybug is a restricted option owing to concerns about residue, interference with natural enemies *etc*. Hence, it is necessary to study the host range, biology and to develop alternative ecofriendly strategies for the management of the mealybug.

The study entitled "Population dynamics, biology and management of mealybug *P. solenopsis* Tinsley (Hemiptera: Pseudococcidae) on okra" was undertaken at the AINPAO (All India Network Project on Agricultural Ornithology) laboratory, Dept. of Agrl. Entomology, College of Horticulture, Vellanikkara during March 2016 – May 2017. The objectives of the study were to study the population dynamics, biology and management of *P. solenopsis* and characterization of its endosymbionts.

To document the host range and natural enemies of *P. solenopsis*, purposive survey was conducted in Thrissur district. *P. solenopsis* was recorded on more than 40 plants, the majority of which belonged to the families *viz.,* Asteraceae, Malvaceae, Solanaceae and Amaranthaceae. As the population of mealybug on okra fields was negligible during survey, study on population dynamics was carried out on major host plants recorded *viz., Sida acuta* Burm.f., *Abutilon indicum* (Link) Sweet, *Hibiscus rosa-sinensis* L.and *Amaranthus viridis* L. Population of mealybug was found to be high during summer (March 2016 to July 2016) and winter seasons (November 2016 to February 2017). Natural enemies recorded included a predator [*Spalgis epius* (Westwood)] and four parasitoids [*Aenasius arizonensis* (Girault), *Anicetus* sp., *Myiocnema comperei* Ashmead and *Prochiloneurus* spp.].

Molecular characterization of mealybug was done to confirm the species identity prior to studies on biology. Mass culturing of mealybug was done on potato sprouts. The mealybugs reproduced through ovo-viviparity and parthenogenesis. Life cycle of female mealybug consisted of three nymphal instars and an adult stage, whereas that of male mealybug consisted of an additional pupal stage along with three nymphal instars. The mean duration of first and second nymphal instars was 4.27 and 6.67 days. The average third nymphal instar duration was 4.8 days. Mean pupal period in male mealybug was 6.8 days. Adult female lived for an average of 38.75 days with pre-oviposition, oviposition and post-oviposition period of 5, 11.5 and 0.5 days respectively. Adult male lived for only an average of 1.7 days. Adult female deposited an average of 171 crawlers with a female to male sex ratio of 1: 0.03. Number of antennal segments varied among each instars. The first and second instar nymphs had six antennal segments, while the third instar nymphs and adult stage possessed seven and nine antennal segments, respectively. Study on damage assessment on okra by *P. solenopsis* showed that second and third instar nymphs produce profuse crinkling and yield loss in okra.

Apot culture experiment was conducted to evaluate the efficacy of entomopathogenic fungi *viz., Paecilomyces lilacinus* (Thom) Samson and *Lecanicillium lecanii* (Zimm.) Zare& Gams each at three different concentrations of 1×10^7, 1×10^8 and 1×10^9 spores ml^{-1}, along with two botanicals *viz.*, NSKE @ 5% and neem oil soap @ 2 %, chemical insecticides *viz.*, buprofezin 250 g a.i. ha^{-1} and thiamethoxam 25 g a.i. ha^{-1} with an untreated control. Thiamethoxam recorded the highest mortality of 75.94 per cent seven days after treatment followed by buprofezin and NSKE with mean mortality of 37.02 and 26.52 per cent, respectively. Entomopathogenic fungi, *L. lecanii* at 1×10^8spores ml^{-1}(21.89%) was on par with *P. lilacinus* at 1×10^7 spore ml^{-1} (20.72%). The mortality of mealybug increased with time lapse after spray application. On the 14^{th} day, highest mortality of 83.54 per cent was recorded in the treatment NSKE which was on par with thiamethoxam (81.74%).

P. lilacinus at 1×10^9spores ml^{-1} and *L. lecanii* at 1×10^8 spores ml^{-1} were found to be the best treatments among entomopathogenic fungi.

Analysis of gut microbiota showed the presence of endosymbiotic bacteria belonging 63 families which constituted 189 species. The major species identified were *Candidatus Tremblaya princeps, Klebsiella* sp., *Pantoea agglomerans* (Ewing and Fife) and *Wigglesworthia glossinidia* Aksoy. Many of the endosymbiotic bacteria are attributed in the survival of insects against toxicants.