

***In-Vitro* Study and Field Surveillance of *Acerophagus papayae* on Papaya Mealybug, *Paracoccus marginatus* in Northern, Sri Lanka**

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Abstract: Among the pests of papaya, *Paracoccus marginatus* is a major pest. It is a highly polyphagous insect pest causing severe yield loss in papaya throughout the year. Many new systemic insecticides are in use; however it becomes a challenge to manage it with just chemical control. This study was carried out to understand the parasitism rate and developmental time of *Acerophagus papayae* with different instars of mealybug, field surveillance of *A. papayae* and the population fluctuation of both *A. papayae* and *P. marginatus* in field. The results were analyzed by using complete randomized design (CRD) by using SAS programme. There was no parasitism of *A. papayae* found in the egg as well as in the first instar of papaya mealybug. Parasitism rate was higher in second instar (56%) than in third instar (50%) and adult mealybug (38%). Lower developmental time was observed in the second instar than third instar and adult. From the field study both *P. marginatus* and *A. papayae* were showed the same pattern of population fluctuations and controlled the *P. marginatus* effectively. Further studies are needed to assess the different natural enemies on papaya mealybug (predators and other parasitoids) and compatibility of bio-rationales and chemical insecticides with parasitoids in field level

Key words: *Paracoccus marginatus* • *Acerophagus papayae* • Parasitoid • Developmental time • Parasitism rate • Population fluctuation • Biological control

INTRODUCTION

Papaya (*Carica papaya*) bears fruits throughout the year, requires less area per plant and provides high income per hectare. It is grown in the home garden of Sri Lanka. Papaw cultivation has thrived in the region with the demand for Red Lady and Sinta varieties for the export market. Papaw is predominantly grown in Jaffna, Kilinochchi, Mullaitivu and Vavuniya, Mannar districts in Northern region of Sri Lanka.

Among the pests, papaya mealybug, *Paracoccus marginatus* is a major pest. It is a highly polyphagous insect pest that can damage large number of tropical and sub-tropical fruits vegetables and ornamental plants [1]. Infestations commonly occur within vegetative shoots or apices, leaves and fruits. Mealybugs form dense colonies, particularly within the shoot and apex, often cause the chemical control of this pest quite difficult. Many new systemic insecticides control has improved; however, these insects have numerous hosts,

it becomes a challenge to manage them with just chemical control [2].

Papaya mealybug parasitoid, *A. papayae* (Hymenoptera: Chalcidoidea: Encyrtidae) is an introduced solitary koinobiont endoparasitoid of papaya mealybug, *P. marginatus*. The choice of host stage is most important in determination of parasitoid's progeny fitness [3].

Knowledge of suitable host stages for parasitism and developmental time of parasitoids lead to better understanding of the Population fluctuations of the host and the parasitoid. Hence, they are important in evaluating and understanding the success of biological control in integrated pest management programs. Therefore, the present study was carried out with the following objectives, study about the *A. papayae*, here the developmental time and parasitism rate was compared with different instars of papaya mealybug and the performance of *A. papayae* was monitored at the same time population fluctuation of both *A. papayae* and papaya mealybug were recorded under field condition.

MATERIALS AND METHODS

Parasitoids are tiny wasps introduced for biological control of insect pest. *A. papayae* Noyes and Schauff recorded as parasitoid on *P. marginatus* Williams and Granara de Willink. There are no enough studies about the parasitism of *A. papayae* to control the *P. marginatus* in Northern region of Sri Lanka. The details of materials used and methodology adopted for this study are described below. This study was carried out in Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Kilinochchi and Regional Agriculture Research Development Centre (RARDC), Kilinochchi, Sri Lanka situated in low country dry zone, receiving on average annual rainfall (over 35 years) 900-1400mm, minimum and maximum relative humidity of 75% - 80%, minimum and maximum day temperature 27-36°C, minimum and maximum night temperature 25-29°C, from January 2016 to May 2016 of experiment period (RARDC, 2016).

In-vitro Study: Egg sacs, nymphs and adults of *P. marginatus* were collected from the papaya fields in different locations in Jaffna and reared on papaya plant in laboratory of Department of Agricultural Biology, University of Jaffna. Population of the papaya mealybug was maintained until completion of the research work.

Twenty-five healthy *A. papayae* were brought from the Horticultural Research and Development Institute (HORDI), Gunnoruwa, Peradeniya, Sri Lanka. Pretreated (soaked in a 1% sodium hypochlorite for 20 minutes, then rinsed with water and air dried) sprouted healthy potatoes (*Solanum tuberosum* L.) were used as food source for rearing mealybugs [4].

Trays with sprouted potatoes were kept inside a dark room at $28^{\circ} \pm 1^{\circ}\text{C}$ and $65 \pm 2\%$ R.H to encourage sprouting. Water was sprayed daily to keep the relative humidity in optimum level. 3-4 newly laid ovisacs of the papaya mealybug were infested on each sprouted potato when the sprouts reach 3-5cm in length.

Mealybug infested potatoes were kept in well aerated plastic containers (8cm in height, 10cm in diameter). 3-4 sprouted potatoes were kept in each container. These containers were placed inside the dark room (to control the movement of 1st instar nymph). After 18-20 days (eggs turn to 2nd instars), five *A. papayae* were introduced into plastic container and 2-3 streaks of 80% honey solution was also offered to parasitoid. The parasitoids were removed after fifth day and placed in another plastic container with second instars of mealybug.

Host Stage Susceptibility: To find out the host stage susceptibility of *A. papayae*, a no-choice test was carried out for egg, first, second, third instars and for newly emerged adult females. A hibiscus leaf with a 3-5 cm long stem was selected as the experimental unit. Petri dishes were prepared as mentioned previously.

The experimental arena for the study was consisted on 9 cm diameter plastic petri dishes. About 0.5 cm diameter hole was made in the bottom of the petri dish using a heated cork borer. A tender Shoe flower leaf with a 3-5 cm long stem was placed in each Petri dish with the stem inserted through the hole at the bottom of the Petri dish. The leaf was placed inside the Petri dish with the stem inserted through the hole [5]. A piece of regiform was also made with holes. Shoe flower stem was inserted through the hole. Below the regiform, a tray was placed with full of water. So that the stem below immersed in water to keep the leaf fresh.

For each mealybug stage, (egg, 1st instar, 2nd instar, 3rd instar, adult female) ten individuals were selected 24 hours before the experiment and placed on each Hibiscus leaf using a camel hair brush. These ten individuals of each mealybug stage were considered as a single experimental unit and five replicates.

A streak of 80% honey solution was also offered inside of the lid [5]. After 24 hours of placing the mealybugs in the Petri dish, a single mated female parasitoid was placed on each leaf and the lid was replaced. The parasitoid was removed after 24 hours and the mealybug retained in same petri dish to continue its development. The mealybug was examined daily. The parasitism rate, developmental time was recorded. The experiment was conducted as complete randomize design with five treatments. Altogether twenty five treatment combinations were used with five replicates.

Field Study: Field study was carried out in Regional Agriculture Research Development Centre (RARDC), Kilinochchi. Four healthy papaw plants without mealybug infestation were randomly selected in the field. Papaw fruits were removed from the plant for easy inspection. Care was taken to avoid application of any chemical pesticide in these experimental locations during the study period. Those plants were introduced with 10-15 mealybug ovi sacs per plant. Plants were covered with parasitoid proof insect nets without disturbing the plants as well as the mealybug colony.



Plate 1: Papaw plant covered with tiny net

After severe infestation (after one month from introduction), the populations of the mealybug was roughly estimated and three plants (three replicates) were introduced with five *A. papayae* into the net. One plant used as control (without parasitoids). After that population of the mealybug and parasitoid, behavior of the parasitoid was observed twice a day. At the same time environmental conditions (temperature, relative humidity) were also recorded.

To monitor the population fluctuations of the parasitoid and mealybug, Papaya leaf samples included four mature leaves pieces were cut at random per tree, totaling twelve leaves cuts from three trees and four leaves pieces from control plant were used to count the mealybug density. Leaves were cut into 4cm * 4cm by using square net. Only the lower surface of the leaves cuts were examined under a microscope. Total numbers of mealybugs per stage of development were recorded.

Twelve (12) leaves cuts were used to average those stages per leaf cut in replicate and four leaves cuts used to average those stages per leaf cut in control in study site. Those leaf cuts were put in the well aerated plastic containers (8cm in height, 10cm in diameter) the rate of two cuts per plastic container with sprouted potato as food source for mealybug in leaf cut. Plastic containers were inspected twice a day and the number of emergence of the parasitoid and the remaining total number of mealybug per stage were recorded. Leaves cuts were taken in two weeks interval for six weeks.

RESULTS AND DISCUSSIONS

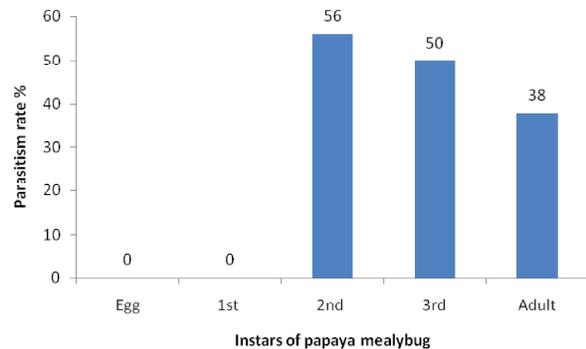


Fig. 1: Parasitism rate of *A. papayae* in different stages of mealybug

Selection of any parasitoid for biological control programmes depend on efficiency of parasitizing various host stages. Fig.1 illustrated that the parasitism rate of *A. papayae* with all stages of papaya mealybug. There was no parasitism found in the egg as well as in the first instar of papaya mealybug. Higher parasitism was observed in the second instar of mealybug with 56%. Amarasekare *et al.*, [6] found that highest percent parasitism in second instar of papaya mealybug. In third instar parasitism was observed similar to second instar with 50%. But parasitism rate was showed lower in adult mealybug than the second instar. This may be due to their defensive behavior and similarly parasitoid needs more time to handle the bigger hosts [7].

Parasitism rate of *A. papayae* in adult mealybug was significantly differed with second instar. But parasitism rate in third instar was not significantly differed with second instar as well as adult mealybug in at 0.05 α .

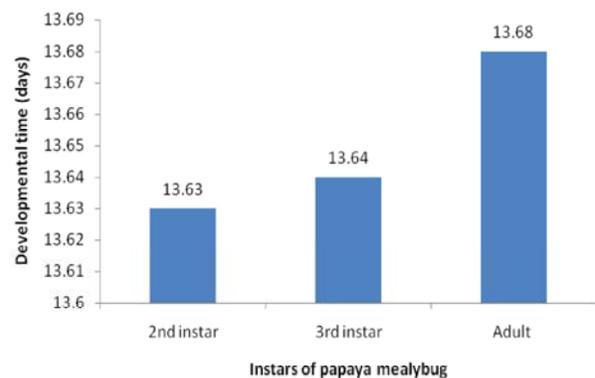


Fig. 2: Developmental time of *A. papayae* in different stages of mealybug

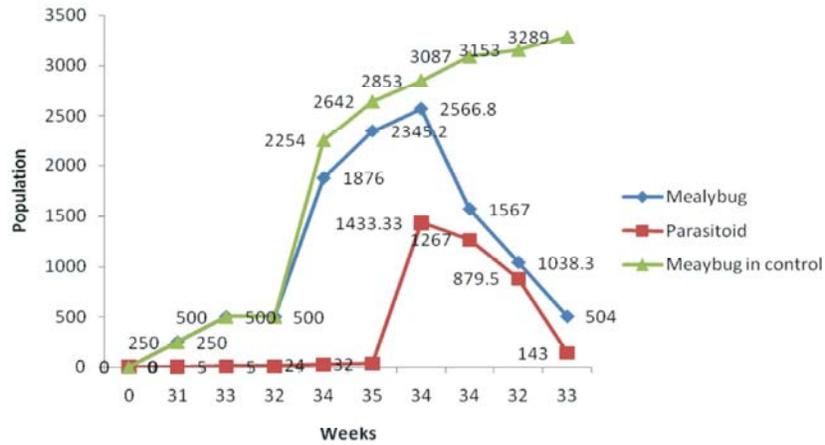


Fig. 3: Population fluctuations of mealybug and *A. papayae*

Figure 2 illustrated that the comparison of developmental time in days of *A. papayae* in different host stages of papaya mealybug. Lower developmental time was observed in second instar than third instar and adult.

But developmental time in the second instar and third instar were not significantly differed at 0.05 α . At the same time it was not significantly differ in third instar and adult too. But it was differed in second instar and adult at 0.05 α .

In control, continuous increasing of mealybug population was observed. Initially it was rapidly increased up to fifth week, but in latter part it was increased with decreasing rate, competition for the food may be the reason for the graph pattern.

In treatment plants, population of mealybug was suddenly increased from four to seven weeks from introduction and reached the peak population because lack of natural enemies as well as plenty of food sources, hence the parasitoid was present, but that was not able to suppress the mealybug population due to the low initial population density of the parasitoid. At the same time parasitoid's population was also increased with mealybug population because there was ample of host and reached the peak population at seventh week.

Then after mealybug population was decreased because of the parasitism of *A. papayae* and the population of parasitoid was also decreased because of the scarcity of the host. Due to that both populations were decreased in same pattern and reached the minimum.

CONCLUSION

There was no parasitism of *A. papayae* found in the egg as well as in the first instar of papaya mealybug.

Parasitism rate was identified as higher in the second instar (56%) than in third instar (50%) and adult mealybug (38%). This may be due to their defensive behavior and similarly parasitoids need more time to handle the bigger hosts and lower developmental time was observed in second instar than third instar and adult.

According to these results the second instar of the mealybug was the most suitable host stage for *A. papaya*. From the field study, population of papaya mealybug was suddenly increased initially because lack of natural enemies as well as plenty of food sources, hence the parasitoid was present, but that was not able to suppress the mealybug population due to the low initial population density of the parasitoid.

At the same time parasitoid's population was also increased with mealybug population density because there was ample of suitable host. After that population of *A. papayae* was decreased with decreasing mealybug population.

These findings would help to manage the papaya mealybug by using locally available parasitoids.

REFERENCES

1. Miller, D.R. and G.L. Miller. 2002. Redescription of *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Coccoidea: Pseudococcidae) including descriptions of the immature stages and adult male. Proc. Entomol. Soc. Wash. 104: 1-23.
2. Amarasekare, K. G. 2007. *Life History of Papaya Mealybug (P. marginatus) and the Effectiveness of Three Introduced Parasitoids (A. papayae, Anagyrus loecki and Pseudleptomastix mexicana)*. ProQuest, 2007.

3. Hagvar, E.B. and T. Hofsvang, 1991. Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. *Biocontrol news and Information*, 12(1): 13-42.
4. Serrano, M.S. and S.L. Lapointe, 2002. Evaluation of host plants and a meridic diet for rearing *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) and its parasitoid *Anagyrus kamali* (Hymenoptera: Encyrtidae). *Florida Entomol.*, 85: 417-425.
5. Mastoi, M.I., A.N. Azura, R. Muhamad, A.B. Idris and Y. Ibrahim, 2014. Parasitism, sex ratio, developmental time and gregariousness of *A. papayae* (Hymenoptera: encyrtidae) on male and female host stages of *P. marginatus* in no-choice situations. *FUUAST Journal of Biology*, 4(1): 43.
6. Amarasekare, K. G., Mannion, C. M. and Epsky, N. D. 2010. Host instar susceptibility, selection and interspecific competition of three introduced parasitoids of the mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae). *Environmental Entomology*, 39(5): 1506-1512.
7. Bertschy, C., T.C. Turlings, A. Bellotti and S. Dorn, 2000. Host stage preference and sex allocation in *Aenasius vexans*, an encyrtid parasitoid of the cassava mealybug. *Entomologia Experimentalis et Applicata*, 95(3): 283-291.