



RESEARCH ARTICLE

LIFE TABLE STUDIES OF *PARACOCCUS MARGINATUS* EFFECT OF ENDOSYMBIONTS ELIMINATION ON LIFE TABLE PARAMETERS OF PAPAYA MEALYBUG *PARACOCCUS MARGINATUS* (HEMIPTERA: PSEUDOCOCCIDAE) ON DIFFERENT HOST PLANTS

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ABSTRACT

The mealybugs were reported to rely on endosymbionts for the essential amino acids which were found to be deficit in the sap of its host plants. Life table describes the mortality and survival patterns of a population is described by life table that provides information on parameters such as the number of survivors, the number of deaths and the life expectancy. The present study analyzes the effect of endosymbiont elimination on the life table parameters of *P. marginatus* on four different host plants viz. papaya (*Carica papaya* L.), eggplant (*Solanum melongena* L.), tapioca (*Manihot esculenta* Crantz) and mulberry (*Morus alba*). Results revealed that net reproductive rate of PMB on all four host plants was significantly reduced by the antibiotic treatment. Irrespective of the host plants, cumulative fecundity of PMB population was high in endosymbiotic population than in aposymbionts; especially it was much reduced on mealybugs of brinjal and tapioca host plants. And also in all the host plants the age of first and 50 per cent oviposition of aposymbiotic population has been postponed and also in papaya and mulberry host plants the total oviposition was prolonged in aposymbiotic population. In aposymbiotic population of PMB on all four host plants, the mean generation time has been prolonged. The outcomes displayed right now give a premise to the advancement of new conventions for tentatively controlling the endosymbiotic microbiota of PMB.

INTRODUCTION

Absolute requirement and obligate vertical transmission of endosymbionts revealed that the insects deprived of these endosymbionts could not find other symbionts in nature. The mealybugs were reported to rely on endosymbionts for the essential amino acids which were found to be deficit in the sap of its host plants (Scarborough *et al.*, 2005). Amino acids present in plant sap are nonessential ones; hence papaya mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae) (PMB) depends on endosymbiotic microorganisms for the supply of essential amino acids and other nutrients, whereby they can live solely on the specialized food source (Moran *et al.*, 2008). PMB in their abdomen it carries a structure called bacteriome, that is packed with bacteriocytes whose cytoplasm is densely populated by endosymbiotic bacteria (Buchner, 1965). The mealybugs harbour a beta-proteobacterial maternally inherited primary endosymbiont, *Tremblaya princeps* and additional gamma-proteobacterial secondary endosymbionts (Fukatsu and Nikoh 1998; von Dohlen *et al.* 2001; Thao *et al.* 2002; Kono *et al.*, 2008; McCutcheon and von Dohlen 2011; Gatehouse *et al.*, 2012).

Cell lysis of these endocellular secondary endosymbionts favours the supposition of direct metabolite supply to the mealybug (McCutcheon and von Dohlen 2011). Disruption of microbial symbiosis demanded by insect pest and symbionts-mediated manipulation of insect pest traits are the two emerging trends in insect pest control. Reports are stating that the complete elimination of endosymbiotic organisms by antibiotics will lead to reduced life span and insect population suppression (Douglas, 2007). Life table describes the mortality and survival patterns of a population is described by life table that provides information on parameters such as the number of survivors, the number of deaths and the life expectancy (Dublin and Lotka, 1937). Hence, our current study analyzes the effect of endosymbiont elimination on the life table parameters of *P. marginatus* on four different host plants viz. papaya (*Carica papaya* L.), eggplant (*Solanum melongena* L.), tapioca (*Manihot esculenta* Crantz) and mulberry (*Morus alba*).

MATERIALS AND METHODS

Culturing of host insect *P. marginatus*: *P. marginatus* were collected from different host plants (papaya, mulberry, brinjal, cassava and congress grass) in farmers filed located at 11°37'35.9"N 78°28'41.1"E. Host plants except papaya were raised in plastic pots of 5kg capacity (containing pot mixture, loamy sand, soil and vermicompost in the ratio of 1:2:1) and

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kept in the metallic cages and the collected mealybugs were released on to their respective host plants using camel hairbrush at the rate of three to five gravid females per plant. Two medium-sized un-ripen papaya fruits were placed in metallic cages, and three to five gravid females per papaya fruit were introduced for multiplication of culture. Colonies of *P. marginatus* was maintained at an ambient environment of 26 ± 2 °C, $60 \pm 5\%$ relative humidity with 12:12 (L: D) photoperiod and mealybugs *en masse* obtained within 25 to 30 days of release.

Development of aposymbiotic mealybug: Aposymbiotic mealybugs were developed by adapting the method of Douglas (1996). The roots of 5 leaf stage host plants were cleaned of excess pot mixture by careful washing in tap water and the plants were transferred to distilled water containing antibiotic (50mg/25ml) and plants in antibiotic-free distilled water will serve as the control. One day later, the second instar mealybug nymphs were released to the plants: those on the ofloxacin-treated plants are described as aposymbiotic mealybugs and those on control plants as symbiotic mealybugs. After 2 days of treatment, all mealybugs were transferred to plants in pots under the standard culture conditions. This treatment was fully effective in disrupting the symbiotic bacteria as indicated by the absence of bacterial colonies in in-vitro isolation experiment. As antibiotic treatment resulted in the reproduction failure of aposymbiotic mealybug, vitamin B (100µg/ml) was supplemented to get next-generation aposymbiotic mealybug population. F1 offspring from populations under control condition and antibiotic-treated population were used for life table studies.

Construction of age-specific life table: In order to construct life tables, single gravid female from the stock culture was released to a new potted host plant and observed for egg-laying. Per day a female mealybug laid about twenty five to twenty nine eggs. Hence, the gravid females were allowed to lay eggs for two days in order to get fifty eggs. On third day after having around fifty eggs, the adult female were removed and with the one-day-old eggs were used for constructing life tables. After hatching, each nymph was examined daily for its survival and fecundity till death of the last individual.

Data analysis: Life and fecundity tables were constructed from the daily account of mortality and fecundity of both endosymbiotic and aposymbiotic PMB population from different host plants following the procedures of Chi and Su (2006). The parameters calculated were as follows:

x:Age class in units of time (days) / developmental stage
 lx: Number surviving individuals at the beginning of age class(x); The number of individuals alive, during a given age interval class as a fraction of an initial population of one
 Lx: Number of individuals alive between age x and x+1; $Lx = (lx + lx+1) \frac{1}{2}$

dx: Number dying during age interval x
 100qx: Percent apparent mortality, $100qx = (dx/lx)100$
 Sx: Survival stage rate within a stage
 Tx: Total number of age x units beyond the age x
 ex: Life expectancy for individuals of age x, $ex = Tx/lx$
 mx: Age-specific fertility, the number of living females born per female in each age interval
 R0: Net reproductive rate, multiplication rate per generation;
 $R0 = \sum lxmx$

Rc: Innate capacity for increase, $Rc = \ln Ro / Tc$
 Rm: Intrinsic rate of natural increase or maximum population growth, calculated by iteration of Euler's equation,

$\sum e^{-rm.x} lxmx = 1$

Tc: Cohort generation time (in day), $Tc = \sum xlxmx / \sum lxmx$

T: Corrected generation time, $T = \ln Ro / rm$

λ: Finite rate of increase, the number of female off-springs female-1 day-1, $\lambda = erm$

Dt: Doubling time, the number of days required by a population to double, $Dt = \ln 2 / rm$

Fixation of survivorship curves was performed using DUD (Doesn't Use Derivative) by adapting the method given by Raltson and Jenrich (1978).

RESULTS

Population and reproductive parameters of *P. marginatus* from our study revealed that in control population (Endosymbiotic) net reproductive rate (No/female/lifetime) was high in PMB reared on papaya (40.48) host plant followed by mulberry (39.06), brinjal (36.38) and tapioca (32.9). In aposymbiotic population, the highest net reproductive rate (No/female/lifetime) of 25.70 was seen in PMB on brinjal host plant followed by on papaya (21.38), mulberry (20.66) and tapioca (20.42). The intrinsic rate of natural increase was 0.14, 0.13, 0.13 and 0.14 in endosymbiotic population and 0.11, 0.10, 0.09 and 0.09 in aposymbiotic population on papaya, brinjal, tapioca and mulberry host plants, respectively. Finite rate of increase (No./Days) in endosymbiotic population was 1.16, 1.14, 1.14 and 1.15, in aposymbiotic population 1.12, 1.10, 1.09 and 1.09 on papaya, brinjal, tapioca and mulberry host plants, respectively.

Time taken to complete one generation by endosymbiotic population was 25.71 days, 26.80 days, 27.53 days and 26.83 days on the host plants papaya, brinjal, tapioca and mulberry, respectively. Whereas, the aposymbiotic population took a prolonged time of 27.24 days, 33.31 days, 33.93 days and 34.96 days, on papaya, brinjal, tapioca and mulberry host plants, respectively to complete one generation. Time for population doubling of endosymbiotic PMB was 4.82, 5.17, 5.46 and 5.07 and of aposymbiotic PMB was 6.17, 7.11, 7.80 and 8.00 days on papaya, brinjal, tapioca and mulberry host plants, respectively. Oviposition starts at the age of 24, 25, 26 and 25 in endosymbiotic PMB and at age of 25, 31, 32 and 28 in aposymbiotic PMB papaya, brinjal, tapioca and mulberry host plants, separately. Oviposition ends at the age of 29, 32, 31 and 32 in endosymbiotic population and in aposymbiotic population it ends at the age of 32, 38, 38 and 34 on papaya, brinjal, tapioca and mulberry host plants, respectively.

Endosymbiotic female mealybugs oviposited for 6, 8, 6 and 8 days and aposymbiotic females for 8, 8, 7 and 7 on papaya, brinjal, tapioca and mulberry host plants, respectively. While, the 50 per cent oviposition occurred in 25.5, 26.5, 27 and 26.5 days in endosymbiotic population and in 27, 33, 33.5 and 30 days in aposymbiotic population on papaya, brinjal, tapioca and mulberry host plants, respectively. Fifty per cent mortality was recorded in 11.5, 13.0, 15.8 and 13.5 days in endosymbiotic population and in aposymbiotic population it was in 13.0, 14.5, 17.3 and 15.3 days on papaya, brinjal, tapioca and mulberry host plants, respectively (Table 1).

Table 1. Population and reproductive parameters of endosymbiotic and aposymbiotic *P. marginatus* on different host plants

Particulars	Papaya		Brinjal		Tapioca		Mulberry	
	E	A	E	A	E	A	E	A
Net reproductive rate (No./female/lifetime) (R0)	40.48	21.38	36.38	25.70	32.90	20.42	39.06	20.66
Intrinsic rate of natural increase (rm)	0.14	0.11	0.13	0.10	0.13	0.09	0.14	0.09
Finite rate of increase (No./days) (λ)	1.16	1.12	1.14	1.10	1.14	1.09	1.15	1.09
Mean generation time (days) (T)	25.71	27.24	26.80	33.31	27.53	33.93	26.83	34.96
Population doubling time (days) (t)	4.82	6.17	5.17	7.11	5.46	7.80	5.07	8.00
Age of first oviposition (days)	24	25	25	31	26	32	25	28
Age of last oviposition (days)	29	32	32	38	31	38	32	34
Length of oviposition (days)	6	8	8	8	6	7	8	7
Age of 50 % oviposition (days)	25.5	27	26.5	33	27	33.5	26.5	30
Age of 50 % mortality (days)	11.50	13.00	13.00	14.50	15.80	17.30	13.50	15.30

E- Endosymbiotic population; A- Aposymbiotic population

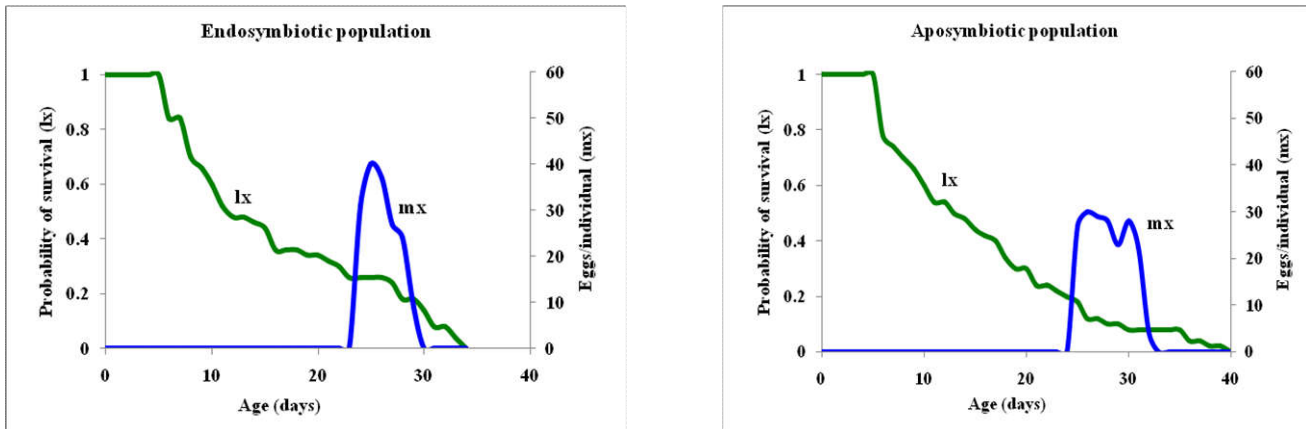


Fig. 1. Life history parameters of endosymbiotic and aposymbiotic populations of *Paracoccus marginatus* on papaya host plant

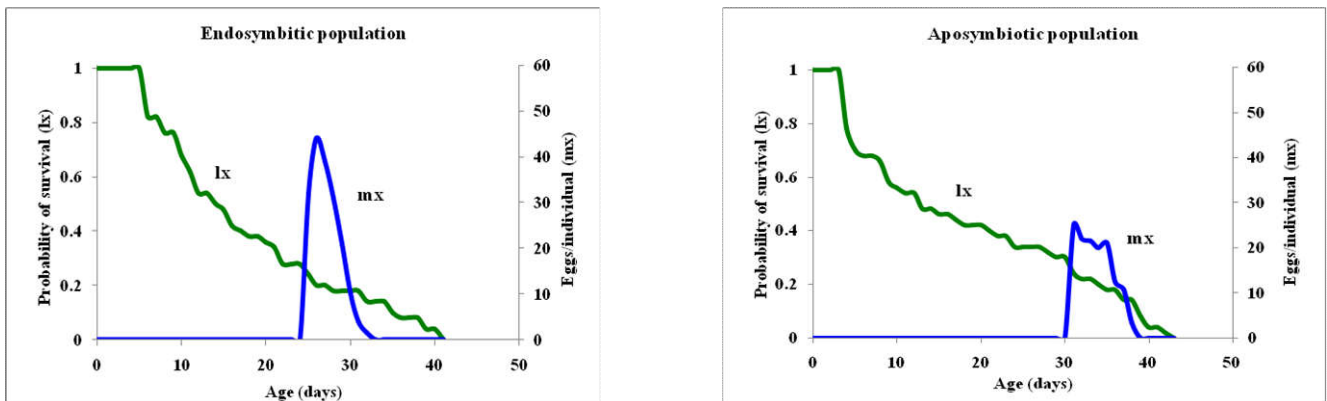


Fig. 2. Life history parameters of endosymbiotic and aposymbiotic populations of *Paracoccus marginatus* on brinjal host plant.

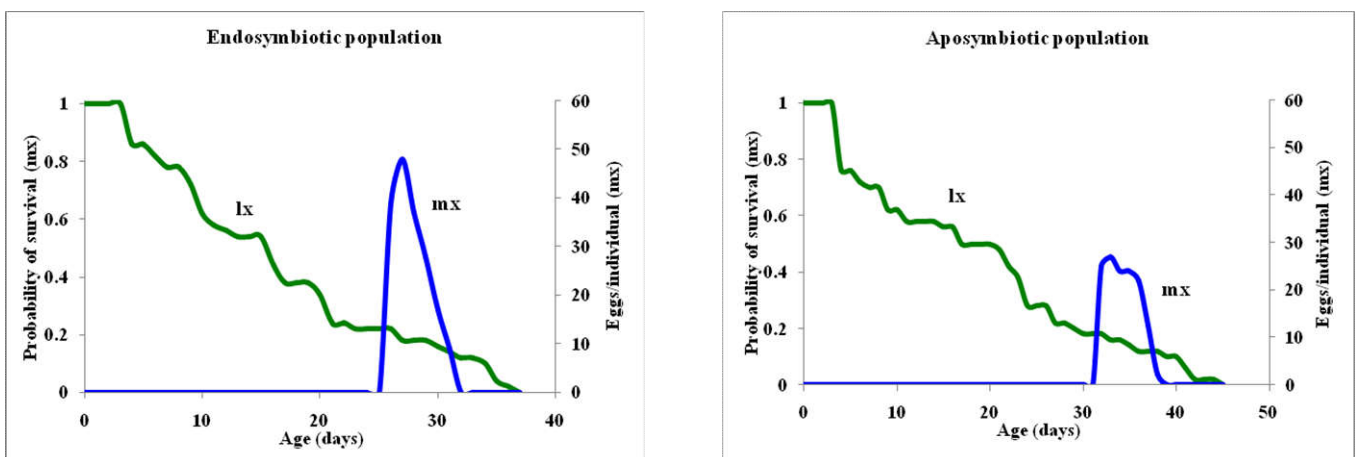


Fig. 3. Life history parameters of endosymbiotic and aposymbiotic populations of *Paracoccus marginatus* on tapioca host plant

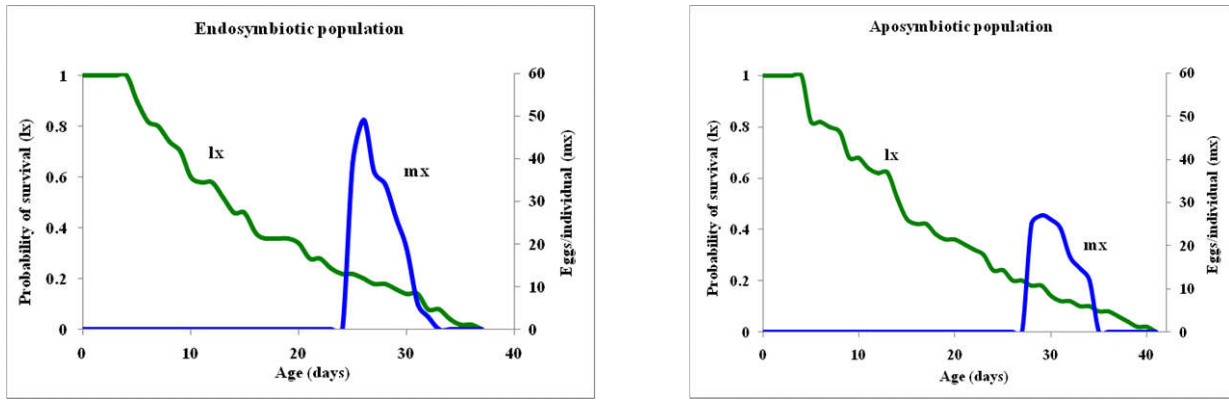


Fig. 4. Life history parameters of endosymbiotic and aposymbiotic populations of *Paracoccus marginatus* on mulberry host plant

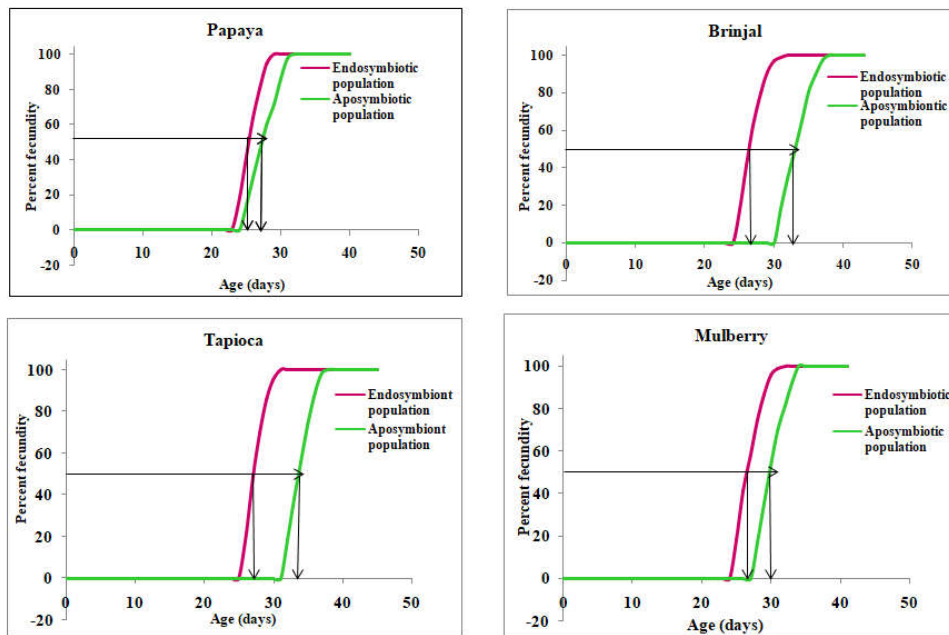


Fig. 5. Cumulative fecundity curve of endosymbiotic and aposymbiotic populations of *Paracoccus marginatus* on different host plants

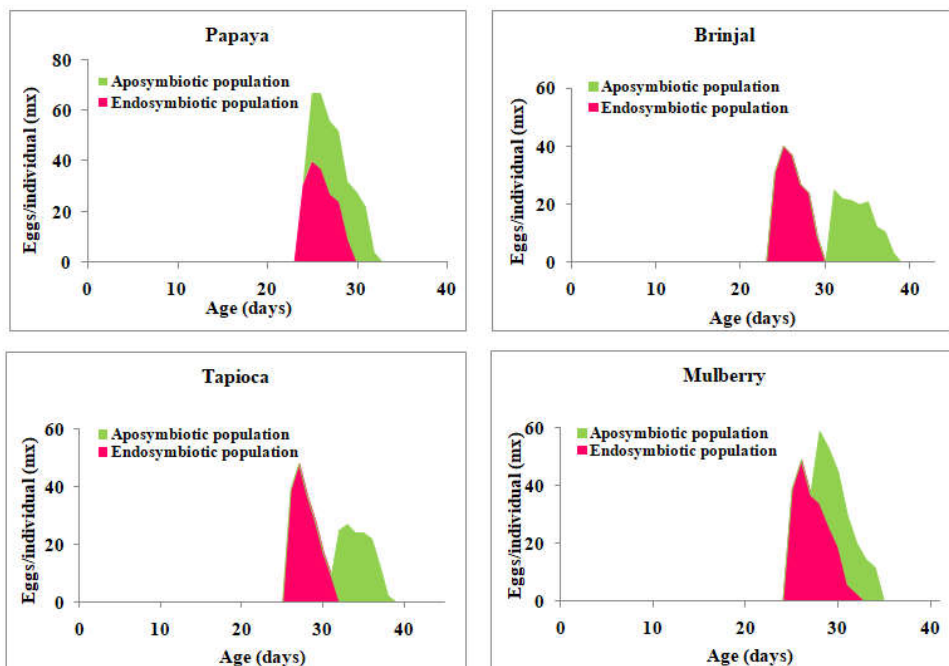


Fig. 6. Age specific fecundity pattern of (mx) F1 offspring of *Paracoccus marginatus* from two populations (under control conditions (endosymbionts) and after antibiotic treatment of their parents (aposymbionts) on different host plants

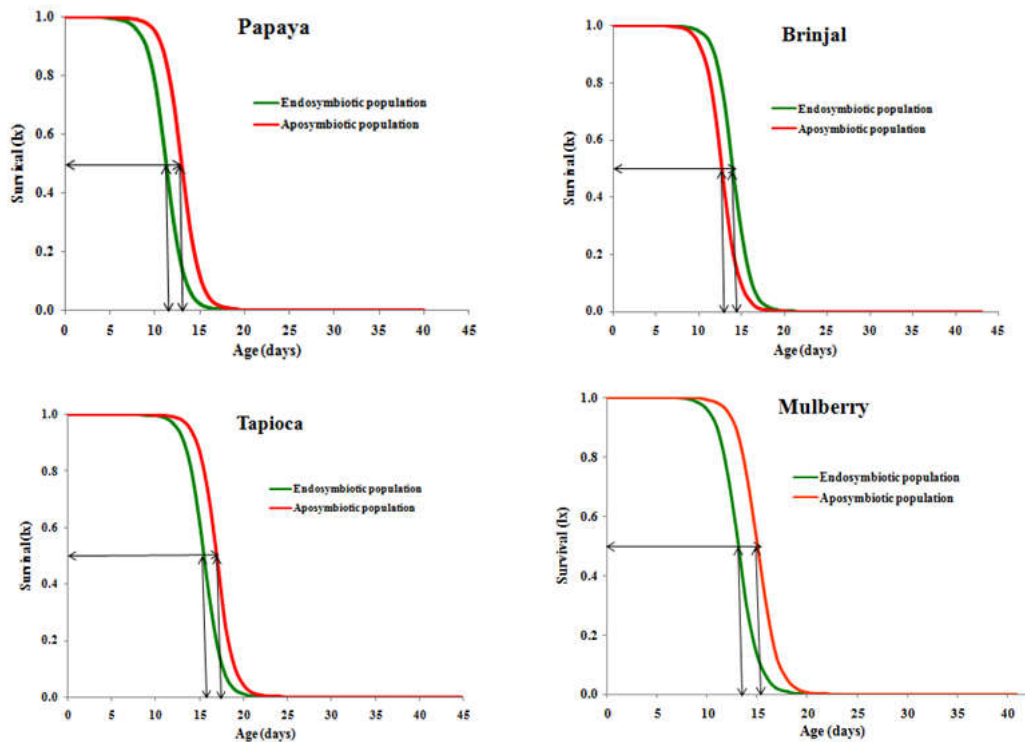


Fig. 7. Age specific survival rate (l_x) of F1 offspring of *Paracoccus marginatus* from two populations (under control conditions (endosymbionts) and after antibiotic treatment of their parents (aposymbionts) on different host plants

DISCUSSION

Deciding the life history of an insect is imperative to comprehend its development, dispersion, and plenitude. In polyphagous insects, life history can differ with the plant species, it benefits from. There were contrasts in the life history parameters of *P. marginatus* raised on four plant species; be that as it may, *P. marginatus* had the option to develop, survive, and reproduce on every one of the four plants. Diverse plant species give distinctive healthful quality and chemical constituents, which can influence the development, proliferation, and survival of an insect. The distinctions saw in the life history of *P. marginatus* might be brought about by nutritive variables, allelochemical mixes, and physical contrasts in leaf structures, albeit none of these elements were read for *P. marginatus* (Amarasekare *et al.*, 2008). In our present study, survival rate and reproduction of endosymbiotic PMB population were found to be high on papaya and mulberry host plants compared to tapioca and brinjal (Fig. 7). Diverse host plant species have been appeared to influence the life history parameters of other mealybug species. Prolonged pre-reproductive period and a higher descendant's generation were watched for *Rastrococcus invadens* Williams raised on various varieties of *Mangifera indica* L. (Boavida and Neuenschwander 1995). Mortality of the of citrus mealybug *Planococcus citri* (Risso) was higher on green than on red or yellow variegated *Coleus blumei* Bellevue (Bentham) plants, and development was quicker and fecundity higher on red variegated plants (Yang and Sadof 1995). The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), could grow similarly well on *Cucurbita pepo* L. as on *C. maxima* (Serrano and Lapointe 2002). There was no distinction in survival, development, and fertility of cohorts of the mealybug, *Phenacoccus parvus* Morrison, when raised on *Lantana camara* L., *Lycopersicon esculentum* Miller, and *Solanum melongena* L. (Marohasy 1997).

Whereas, *Gossypium hirsutum* L., *Ageratum houstonianum* Miller, and *Clerodendrum cunninghamii* Benth were recognized as less appropriate host plants for the improvement of *P. parvus* contrasted and *L. camara* (Marohasy 1997). Irrespective of the endosymbiotic and aposymbiotic PMB population on different host plants mortality was more at first nymphal instars (Fig. 1 to Fig. 4). A loss of 17 to 18% of the first instars was additionally seen on hibiscus, acalypha, and parthenium (Amarasekare *et al.*, 2008). The loss of first-instar *P. marginatus* might be brought about by the movement of crawlers (first instars) away from the leaf tissues looking for appropriate host tissue and tumbling off the plants. This movement was seen on all host species. Starter considers demonstrated that the crawlers of *P. marginatus*, when unstuck from the leaf, were not have the option to survive, except if they moved back or were set back on the leaf. At last, the low survival rate of eggs and first-instar nymphs was resulted in the low cumulative survival rate of *P. marginatus*. This is the first report that antibiotic treatment can influence the wellness of the PMB population. PMB from different host plants like papaya, brinjal, tapioca and mulberry may have a different endosymbiotic profile in it. Leonardo and Muiru (2003) and Tsuchida *et al.* (2004) indicated that populations of the pea aphid, *Acyrtosiphon pisum*, related with various host plants or found in various topographical areas may have various supplements of symbionts. The present study revealed that net reproductive rate of PMB on all four host plants was significantly reduced by the antibiotic treatment (Fig. 1 to Fig. 4). Irrespective of the host plants, cumulative fecundity of PMB population was high in endosymbiotic population than in aposymbionts; especially it was much reduced on mealybugs of brinjal and tapioca host plants (Fig. 5). And also in all the host plants the age of first and 50 per cent oviposition of aposymbiotic population has been postponed and also in papaya and mulberry host plants the total oviposition was prolonged in aposymbiotic population (Fig. 6).

In aposymbiotic population of PMB on all four host plants the mean generation time has been prolonged (Fig. 7). Prolonged developmental times and higher mortality in the aposymbiotic group has also been reported by Kafil *et al.* (2013) in the Sunn pest, *Eurygaster integriceps*. The expanded development time and diminished survival rate of *Arsenophonus*-cleared *Ommatissus lybicus* are reliable with the significant role of *Arsenophonus* in synthesizing essential nutrients for *O. lybicus* (Karimi *et al.*, 2019). The impact of antibiotics on endosymbionts relies exceptionally upon the types of endosymbiont (Ahmed *et al.* 2010), antibiotic dose, and duration of treatment. In addition, the life spans of uninfected whiteflies both males and females expanded as contrasted with those contaminated with *Arsenophonus* (Raina *et al.*, 2015). These outcomes connect with prior works by Ruan *et al.* (2006) and Fang *et al.* (2014) on other optional endosymbionts *Hamiltonella* and *Cardinium*, separately wherein they have detailed that auxiliary endosymbiont infection diminishes the wellness and competitiveness of whitefly host. There have been different examinations which have demonstrated beneficial impacts on development and survival of whiteflies as a result of essence of explicit auxiliary endosymbiont (Su *et al.*, 2013; Xue *et al.*, 2012; Himler *et al.*, 2011; Costa *et al.*, 1997). The expulsion of auxiliary endosymbionts from *B. tabaci* may deliver both positive and ominous impacts on the wellness of the host insect (Ruan *et al.*, 2006). In contrary to the present finding, *Arsenophonus* free whiteflies have higher wellness than the *Arsenophonus* tainted ones (Raina *et al.*, 2015). Wilkinson (1998) inspected contemplates on endosymbionts of aphids and made the recommendation that treatment of aphids with antibiotics to wipe out the advantageous microscopic organisms has no direct malicious impacts on aphid biology.

Conclusion

Late investigations have indicated that obligate symbionts, for example, *Buchnera* as well as facultative symbionts, for example, *Serratia*, *Hamiltonella* and *Regiella* assume a significant biological role for their host insects, especially in explicit environmental settings. For instance, resilience to high temperature is presented by *Serratia* and *Hamiltonella* (Montllor *et al.*, 2002, Russell and Moran, 2006); protection from parasitoid wasps is brought about by *Hamiltonella* and *Serratia* (Oliver *et al.*, 2003, 2005); widening of host plant range is empowered by *Regiella* (Tsuchida *et al.*, 2004); protection from parasitic organisms is given by *Regiella* (Scarborough *et al.*, 2005); and enlistment of winged transform and sexual age is adjusted by *Regiella* (Leonardo and Mondor, 2006). Insects speak to most of the biodiversity in the global biological system and the more significant part of all insect species is assessed to be in relationship with endosymbiotic microorganisms (Jeyaprakash and Hoy, 2000; Werren and Windsor, 2000). We are just currently getting aware of the general significance of unrecognized microbial partners that may significantly influence different life-history attributes of these insects. The outcomes displayed right now give a premise to the advancement of new conventions for tentatively controlling the endosymbiotic microbiota of PMB and different insects, in this way altogether improving our comprehension of insect ecology and evolution.

Conflict of interest: The authors declare that there is no conflict of interest.

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Abbreviations

°C - degree Celsius

L: D - Light : Dark µg/ml – Microgram per milliliter

mg/ml – Milligram per milliliter

PMB - Papaya mealybug

% - Per cent

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