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Host preference analysis of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on three different host plants.

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Abstract
The cotton mealy bug *Phenacoccus solenopsis* Tinsley is sap sucking polyphagous pest over 154 plant species of vegetables, fruit and ornamentals including cotton in India and worldwide. Among vegetable plant species, brinjal (*Solanum melongena* L.), tomato (*Solanum lycopersicum* L.), okra (*Abelmoschus esculentus*) etc., are severely damaged. Present studies were carried out to investigate the impact of these three host plant species on the life history of the mealybug in the laboratory. Age, stage specific and female fertility table data were used for this analysis. Okra has been found most suitable host among the tested plant species. Shortest development period (52 days), longest fertility period (21 days) and natality (163 eggs/female) were recorded on okra. In addition, highest values of intrinsic rate of increase (r_m) (0.09569±0.002 day−1), the finite rate of increase (λ) (1.10±0.002 day−1), and net reproductive rates (R_0) (38.60±2.35 offspring per individual) were also recorded on okra. On the other hand, the longest mean generation time (T) (48.95±2.88 days) and doubling time (DT) (11.55±1.25 days) were observed when *P. solenopsis* was reared on tomato. The tomato was the least preferred host for *P. solenopsis*. This variation in host preference can be utilized in predicting the dynamics of the *P. solenopsis*, and in determining the timing of pesticide application as well as release timing for natural enemies to achieve better control of this pest. Further, okra can be used as trap crop to facilitate effective management of this pest.

Keywords: *Phenacoccus solenopsis,* host preference, tomato, brinjal, okra, life-table

Introduction

The cotton mealy bug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is one the most destructive sucking pest of cotton, okra, brinjal, tomato, legumes, ornamentals, etc. It was reported firstly from New Mexico, USA in 1992 (El Aalaoui and Sbaghi) [8] and further expanded its boundaries to Africa, Europe, North and South America, Australia, Asia, Oceania, Mediterranean basin, Canary-islands, and many middle eastern countries including Saudi Arabia (Zhou *et al.* Fand and Suroshe; Gebregergis; Katbeh Bader and Al-Jboory; Ricupero *et al.* El Aalaoui and Sbaghi) [42, 9, 10, 17, 29, 8].

It inflicts direct as well as indirect damage to the crops, ultimately resulting into serious yield losses. In direct mode of infestation, the adults, and nymphs of mealybug feeds on phloem and suck the sap from all the plant parts including root, young shoots, leaves, flowers, and fruits (Jhala *et al.* Nagrare *et al.*) [15, 25]. Excessive de-saping results into malformed growth of the plant which is characterized by yellowing of the plant parts, curling, and crinkling of leaves, which further leads to weakening and defoliation and pre mature fall of flowers and fruits and in extreme case leads to the death of the plant (Bragard *et al.*) [3] Whereas, indirect mode of infestation result in production of honey dew which encourages growth of sooty mold on leaves affecting the process of photosynthesis in the plant. Presence of sooty mould on fruits makes them unmarketable (Joshi *et al.* Lysandrou *et al.*, Sahito *et al.* Nabil) [10, 21, 31, 24]

The Asian countries such as India and Pakistan, have suffered severe economic losses in cotton caused by exotic mealybug *P. solenopsis* with yield-losses of 30–60% (Nagrare *et al.* Fand and Suroshe) [25, 9]. In India, apart from cotton, many other plant species viz., vegetables (okra, brinjal and tomato) are also infested including uncultivated plants, weeds, horticultural plants, and ornamentals plants (Vennila *et al.* Halder *et al.* Shahid *et al.*) [38, 14, 34].

Since its introduction in the country, this pest has spread fast and it is being continuously reported from newer areas, both from cotton and non-cotton growing areas.

During survey conducted by various authors in India, incidence of this pest has been recorded on vegetables as alternate hosts which is a cause of concern. Some of the important vegetables found infested with higher incidence levels includes tomato, brinjal, potato, okra, cluster bean etc (Nagare *et al.* Venilla *et al.* Halder *et al.*) [25, 38, 14].

The *P. solenopsis* being polyphagous insect pest, knowledge of its alternate host plants with its present status is necessary for devising effective management strategies. Studying, growth parameters, survival and mortality and reproduction can be crucial in determining the host preference of a pest. The present investigation determines the host suitability and preference of *P. solenopsis* among vegetable crops plants.

Materials and Methods

Insect culture

Nymphs and adults of unknown age of *P. solenopsis* were randomly collected from the okra plants cultivated in the field of Department Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India. These developing stages were then released in Petri dishes (9.0×2.0 cm) lined with blotting paper and fresh okra was provided as food. These petri plates were kept at 24±1 ºC; 65±5% RH; 10L: 14D in (BOD) incubator. These were further allowed to feed and reproduce to establish new colonies of cotton mealybug. In order to maintain hygienic conditions, fresh excised okra was supplied daily until nymphs reached up to an adult stage. For oviposition, newly emerged adults were placed in Petri dishes (9.0×2.0 cm) containing fresh okra as food. The laboratory reared adults were used in various experiments as below.

Life cycle studies of *P. solenopsis*

Eggs of the same age of *P. solenopsis* were collected from the stock culture to make a batch of 10 eggs and replicated 10 times to make a cohort (~100). It was replicated three times at 24 ºC temperature. The observations were made on incubation period and counting of hatched and un-hatched eggs was done. Un-hatched eggs were removed from the experiments. After hatching fresh leaves and fruits of other host plants *i.e*., brinjal, okra and tomato were provided to nymphs. The method of rearing of *P. solenopsis* at each constant temperature and on different host plants was same as described above. Daily observation of survival and mortality of each stage *viz*., 1st, 2nd and 3rd instar was recorded. Survivorship of pre-pupal and pupal period of male was also recorded. One pair of *P. solenopsis* was transferred in a Perti dish (9.0×2.0 cm) containing fresh leaves/ fruits of different host plants as food for oviposition. This same experiment was replicated 10 times for all the host plants at constant temperature 24º, 27º and 30 ºC. In order to maintain the freshness of the leaf, leaf petiole was wrapped with wet cotton swab. Eggs laid by female in every Petri dish, were counted daily to record natality rate (m_x). This was continued till the death of all the females. Thus, Age-specific, Stage-specific, fecundity and life indices tables were established based on Deevey [6], Harcourt [13] and Southwood [35] as follows:-

(a) Age specific life-table

x = Age of insect (days)

l_x = Number of surviving at the beginning of each age *x* out of 100

d_x = Number of dying during age interval *x* out of 100

$100q_x$ = Percentage mortality, computed through following equation:

$$100q_x = [d_x / l_x] \times 100$$

e_x = Expectation of life remaining for individuals of age *x*

$e_x = T_x / l_x$

To obtain e_x, two other parameters L_x and T_x were also computed as below:

L_x = Number of surviving between age x and x+1 and calculated by the equation:

$$L_x = l_x+1 (x+1)/2$$

T_x = Total number of individuals of *x* age units beyond the age x and obtained by the equation:

$$T_x = l_x + (l_x + 1) + (l_x + 2)\ldots\ldots\ldots+l_w$$

Where, l_w = the last age interval

(b) Stage specific life table

To build the stage specific life table of *P. solenopsis,* the survival and mortality was taken into contemplation. The experiment was designed as same as designed earlier with same number of insect and with same replications at constant temperature (24º, 27º and 30 ºC) on okra, brinjal and tomato. From age specific survival and mortality life table cumulative data on survival of various stages: eggs, larvae, pre-pupae, pupae and adults were taken for computing various parameters as given below:

Apparent mortality

It provides the data on number of insects dying as percentage of number entering a particular stage and was calculated by using the formula:

$$\text{Apparent mortality } (100q_x) = [\,dx / Ix] \times 100$$

Where, x = Age of insects in days

Ix = Number surviving at the beginning of each interval *x* out of 100

dx = Number dying during the age interval *x* out of 100

Survival fraction

For calculating the survival fraction (S_x) of each stage data was obtained from apparent mortality and calculated using the equation:

S_x of particular stage = [I_x of subsequent stage] / [I_x of particular stage]

Mortality survival ratio

It is the increase in population that would have occurred if the mortality in the stage, in question had not occurred and was calculated as follows:

MSR of particular stage = [Mortality in particular stage] / [I_x of subsequent stage]

Indispensable mortality

This mortality would not be there in case the factor(s) causing it is not allowed to operate. The equation is:

IM = Number of adults emerged × M.S.R. of particular stage

Generation mortality (K – factor) analysis

It is the key factor which is responsible for increase or decrease in number from one generation to another and computed as the difference between the successive values for "log I_x". The total generation mortality was calculated by adding the k-value of different developmental stages of insect, which is designated as "K" (Southwood) [35].

$$K = k_E+k_1+k_2+k_3+k_4+k_p$$

Where, k_E, k_1, k_2, k_3, k_4, k_p are the k-values at egg, 1st, 2nd, 3rd, 4th instar and pupal stages.

(c) Fecundity and life indices

Fecundity was buildup with the following assumptions;

1) Survivorship rates are assumed to be the same for both sexes, as it is not possible in most of the cases to identify the sexes.
2) Sex cannot be identified at egg stage. Therefore, a sex ratio of 1:1 is considered in each batch of eggs.

For adults, the survival rate from birth of age *x* (I_x), potential fecundity (P_f), total number of offspring produced at age, *x* and m_x (female offspring produced at age *x*) were calculated according to Birch (1948). From these data, the intrinsic rate of increase (r_m, females/female / day), mean generation time (T_c) and doubling time (DT, days) were estimated using excel sheet fitted with the following formulas. The table consists of the following column:

x = Pivotal age (days)

lx = Number of female alive at the beginning of age interval *x* (*x*-pivotal age) as fraction of an initial population of one (Birch) [2].

m_x = Average number of eggs laid per female in each age interval assuming 50:50 sex ratio and computed as or mean number of female offspring produced in a unit time by a female age (*x*)

$$m_x = N_x/2$$

Where, N_x = Total natality per female offspring in each age

Beside m_x total number of offspring in each age interval, *i.e*., female eggs laid in age interval (*x*). A number of parameters were also computed from the age specific and fecundity tables, which include:

Potential fecundity (pf)

It is defined as the total number of eggs laid by an average female in her life span and can be calculated by adding up the age specific fecundity (mx) column and measured in female/ female/ generation:

$$p_f = \sum m_x$$

Net reproductive rate (R_0)

It is also known as "carrying capacity" of the average insect under defined environmental conditions. The information on the multiplication rate of a population in one generation is obtained from it or the number of times a population will multiply per generation. It is calculated by the formula:

$$R_0 = \sum l_x.m_x$$

Intrinsic rate of increase (r_m)

It is known as biotic potential and can be defined as the instantaneous rate of increase of population in a unit time under a set of ecological conditions. It can be calculated using the method of Lotka [20].

$$\sum l_x\, m_x.e^{-r.mx} = 1$$

Finite rate of increase (λ)

It is determined as the number of times the population will multiply itself per unit time (measured in unit of females/ female/ day) and is obtained from $\lambda = e^r$

Mean length of generation (T_c)

It is defined as the mean period between the birth of the parent and the birth of off spring. This period is a weighed approximate value or is the mean period over which progeny are produced and estimated by the formula:

$$T_c = l_n\, R_0/r$$

Corrected generation time (r)

It is defined as the period from birth of individuals to birth of offspring and calculated as:

$$\tau = l_n\, R_0/r_m$$

Doubling time (DT)

It is defined as the time required for the population to double its number and is calculated as:

$$DT = l_n\, 2/r_m$$

In order to maintain the above life table parameters, pre-oviposition, oviposition and post-oviposition periods and adult longevity and fecundity, were also estimated at above mentioned constant temperature regimes.

Sex ratio analysis

Newly emerged adult of *P. solenopsis* was collected from each of the three replications of their three different temperature regimes on respective host plants i.e., okra, brinjal and tomato. Sex of *P. solenopsis* can easily be identified based on presence of wings on male. Sex ratio was analysed as the proportion of offspring that are male and can be calculated according to Wilson and Hardy [42] with the help of formula:

Sex ratio = ♂♂ / (♀♀ + ♂♂)

Statistical analysis

The uniformity of all the variance was tested by using Bartlett's test prior to computing the one-way analysis of variance (ANOVA). The difference in r_m and other life table parameters were checked for significance by using the Jackknife method (Maia De) [23]. With the help of computer program Jackknife pseudo-value were calculated (La Rossa and Kahn) [19] and the mean Jackknife Pseudo-value for each treatment was subjected to one way analysis of variance

(ANOVA). Tukey's HSD post-hoc test was used to compare r_m and other life table parameters at various constant temperatures on each host plants. Statistical analysis and graph were done using the language program R 2.10.1 (R Development Core Team, 2010).

Results

Immature development

The outcomes from the present study showed that cotton mealybug, *P. solenopsis* completed its life cycle on all three tested host plants (okra, brinjal and tomato). The incubation period took only one day without any significant difference when reared on different host plants (Table 1). The sexual dimorphism can be seen from second instar and the number of instars vary between males and females. In this investigation, only male and female instar were not segregated and studied as four nymphal instars (Table 1). The immature development for various nymphal instar varied on all the host plants and significantly longest duration was recorded on tomato followed by okra and the shortest on brinjal (df=2, $p<0.000$). The first instar duration was recorded 07.65±0.52 days on okra, 07.25±0.79 days on brinjal, and 08.98±0.59 days on tomato (F= 91.42, df=2, $p<0.000$); second instar took 08.65±0.72 days on okra, 06.57±0.89 days on brinjal, and 08.78±0.54 days on tomato (F=87.44, df=2, $p<0.000$); third instar/prepupa completed in 06.89 ± 0.44 days on okra, 06.75±0.54 days on brinjal, and 08.56 ± 0.53 days on tomato (F=92.32, df=2, $p<0.000$); fourth nymphal instar/pupa lasted for 8.89 ± 0.34 days on okra, 07.20±0.34 days on brinjal, and 09.89 ± 0.85 days on tomato (F=114.88, df=2, $p<0.000$) (Table 1). The total immature development of *P. solenopsis* was calculated separately for males and females. It was observed longest when reared on tomato with a significant difference (males- F=138.64, df=2, $p<0.000$; females- F=118.22, df=2, $p<0.000$) (Table 1).

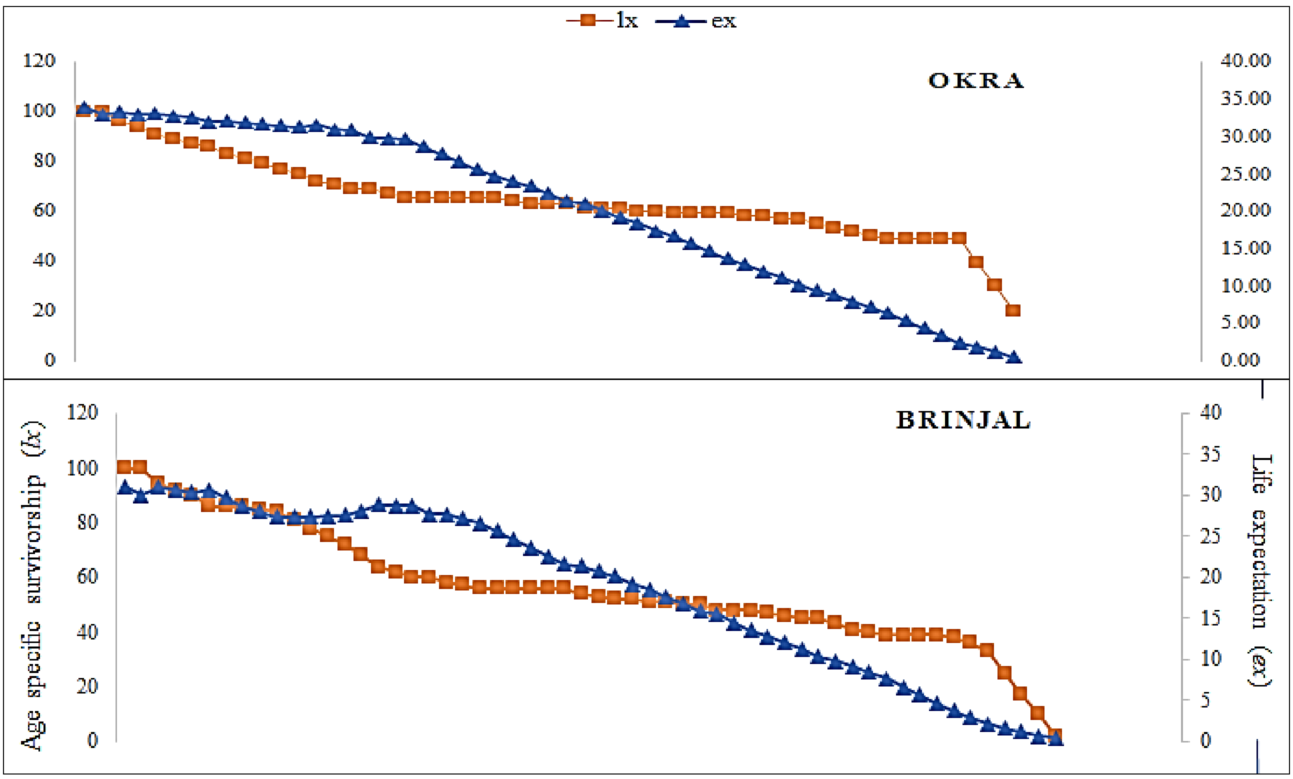
Table 1: Development of immature stages of *P. solenopsis* reared on various host plants.

Host plants	Eggs	L_1	L_2	L_3	L_4*	TID**(M)	TID**(F)
Okra	1.50±0.05a	07.65 ± 0.52a	08.65 ± 0.72a	06.89 ± 0.44a	8.89 ± 0.34a	33.58±1.25a	24.69±1.76a
Brinjal	1.50±0.05a	07.25±0.79b	06.57 ± 0.89b	06.75±0.54b	07.20±0.34b	29.27±2.54b	22.07±2.15b
Tomato	1.50±0.05a	08.98 ± 0.59c	08.78 ± 0.54c	08.56 ± 0.53c	09.89 ± 0.85c	37.71±1.35c	27.92±1.45c
df =	*2,2*	*2,2*	*2,2*	*2,2*	*2,2*	*2,2*	*2,2*
F =	88.26	91.42	87.44	92.32	114.88	138.64	118.22
P <	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Means followed by same letter in column are not significantly different (p<0.05) by Tukey's HSD
*Crawlers that developed into males had a fourth instar
**TID = Total immature development

Age and stage specific survival and mortality

When reared on different host plants, the age specific survival (*lx*) and life expectation (*ex*) curve for *P. solenopsis* exhibited a clear variation (Fig 1). The *lx* was recorded longest (62 days) on tomato and shortest (52 days) on okra (Fig 1). Whereas the life expectation (*ex*) was calculated maximum on okra (33.92) minimum on tomato (24.94). Early mortality in *P. solenopsis* population (in first instars) was also seen highest when reared on tomato (fig 2). This variable rate of development among individuals and an overlapping between different stages was observed on all the host plants tested in this study. The chances of the eggs of *P. solenopsis* to become adults were highest in case of okra.



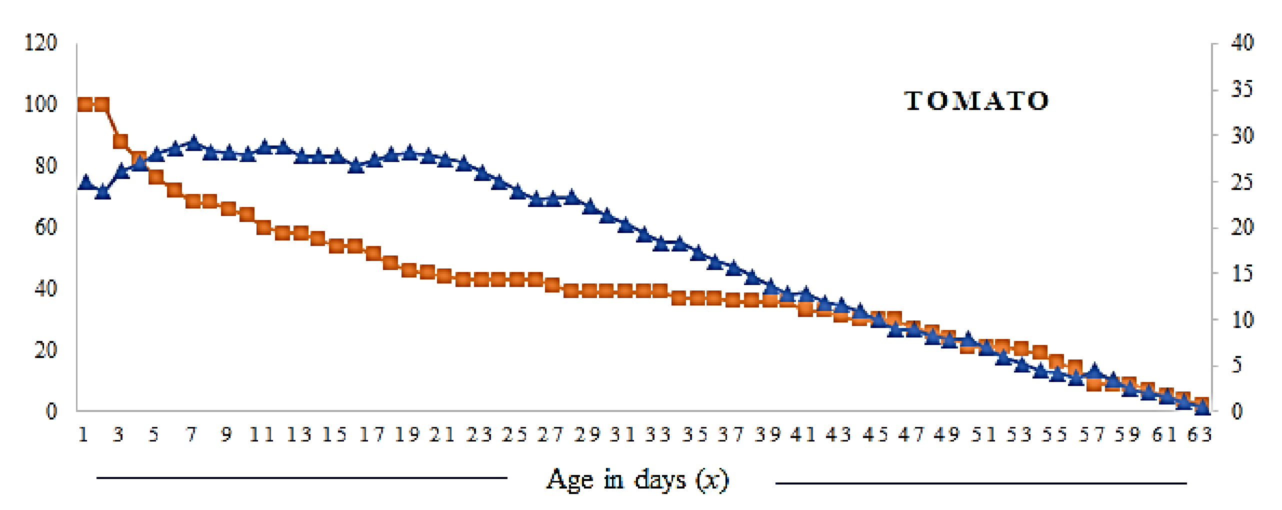
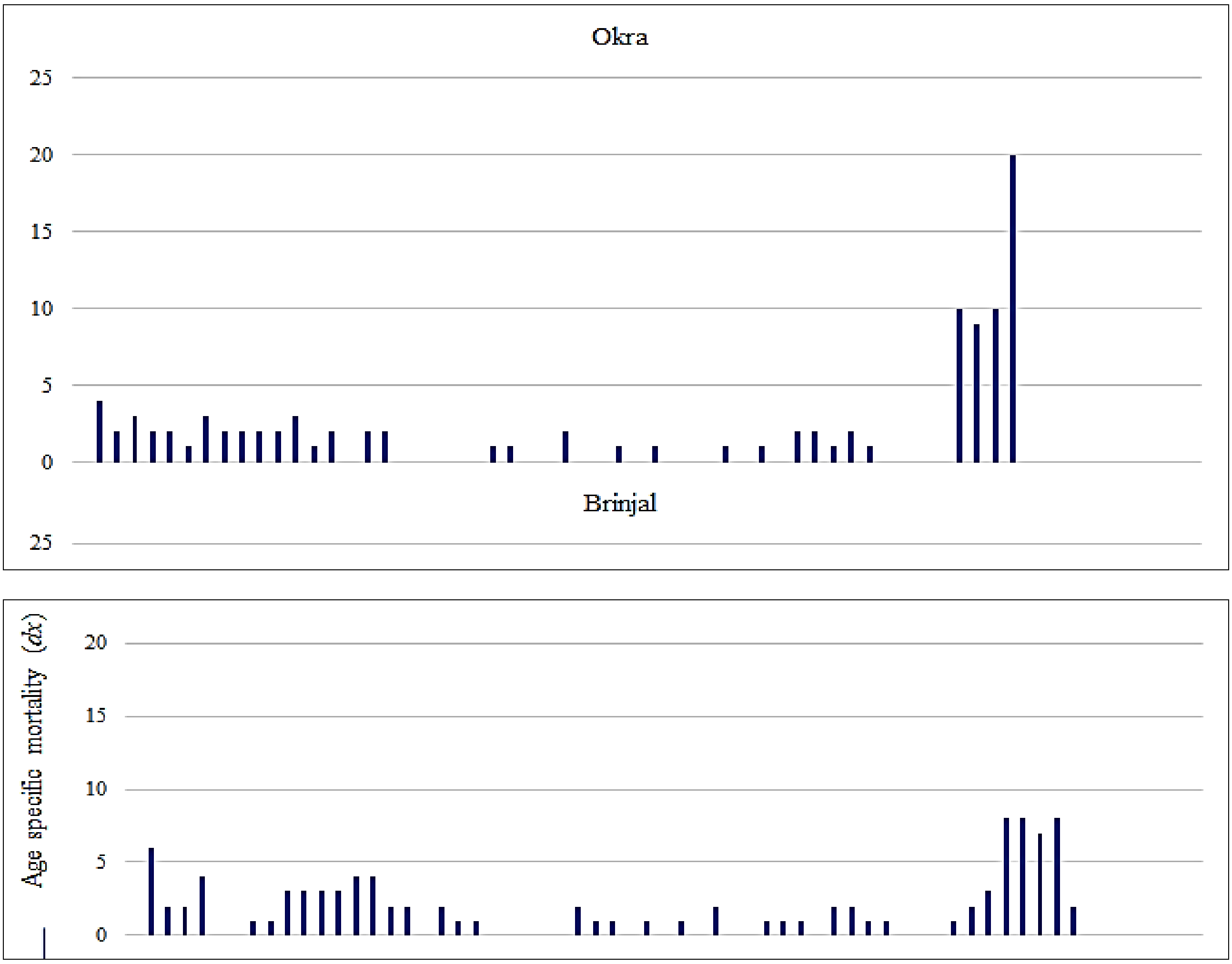


Fig 1: Age specific survival and life expectancy of *P. solenopsis* on different host plants.

As far as different life stages of *P. solenopsis* were concerned, the maximum stage specific mortality (*dx*) was recorded at egg stage on the tested host plants (Table 2). The highest *dx* (44 individuals) at egg stage was observed on tomato and minimum (23 individuals) on okra (Table 2). The total generation mortality (k-value) at first and second instar stage was calculated highest on tomato followed by brinjal and lowest on okra whereas at third instar stage, the lowest k-value was obtained on brinjal (Fig. 3). The age and stage survival and mortality rate represents the probability that a newly hatched egg will survive to adult stage (Seyfollahi *et al.* El Aalaoui and Sbaghi) [8, 32].



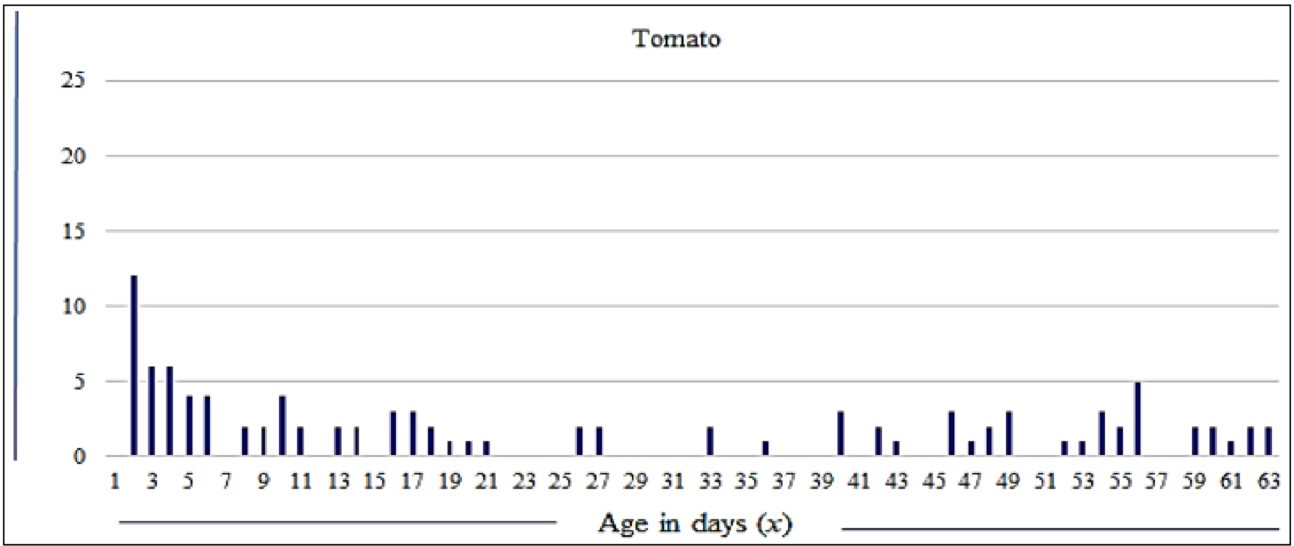


Fig 2: Age specific mortality of *P. solenopsis* on different host plants.

Table 2: Stage specific survival and mortality statistics of *P. solenopsis* on different host plants.

Stages (*x*)	No. surviving at beginning of stage	No. of dying at each stage	Apparent mortality	Survival Fraction	Mortality Survival ratio	Indispensable mortality	log lx	k-value
	lx	dx	100 qx	Sx	MSR	IM		
Okra								
Egg	100	0	0.00	1.00	0.00	0.00	2.00	0.00
I instar	100	23	23.00	0.77	0.30	8.96	2.00	0.11
II instar	77	12	15.58	0.84	0.18	5.54	1.89	0.07
III instar	65	13	20.00	0.80	0.25	7.50	1.81	0.10
Adult	52	0	-	-	-	-	1.72	-
Brinjal								
Egg	100	0	0.00	1.00	0.00	0.00	2.00	0.00
I instar	100	28	28.00	0.72	0.39	11.67	2.00	0.14
II instar	72	16	22.22	0.78	0.29	8.57	1.86	0.11
III instar	56	5	8.93	0.91	0.10	2.94	1.75	0.04
Adult	51	0	-	-	-	-	1.71	-
Tomato								
Egg	100	0	0.00	1.00	0.00	0.00	2.00	0.00
I instar	100	44	44.00	0.56	0.79	23.57	2.00	0.25
II instar	56	15	26.79	0.73	0.37	10.98	1.75	0.14
III instar	41	5	12.20	0.88	0.14	4.17	1.61	0.06
Adult	36	0	-		-	-	1.56	-

*Survival value of fourth instars is not available due to the difficulty in separating sexes at the third instar

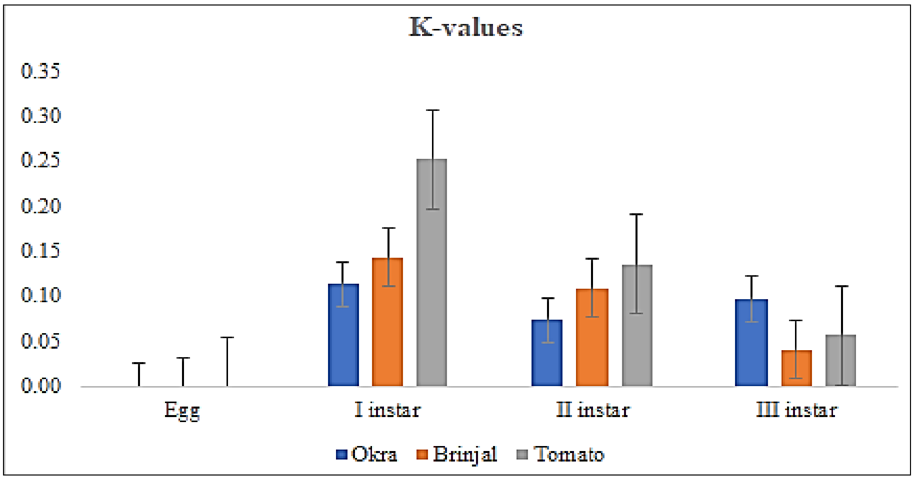


Fig 3: K-values for different life stages of *P. solenopsis* on different host plants.

Female fertility parameters

Result of present studies indicated that all the tested host plants influenced the reproductive parameters of *P. solenopsis*. The highest sex ratio (proportion of females) was obtained when reared on okra (0.62) followed by brinjal (0.59) and lowest on tomato (0.58) (F=54.22, df=2,

p<0.0001) (Fig. 5). The age-specific female survival rate (*lx*) and age-specific fertility (*mx*) have been found host dependant in this study. The highest peaks of female fertility (*mx*), and longest female survival (*lx*) was found on okra (Fig 4). The highest value of intrinsic rate of increase (r_m) (0.09569±0.002 day−1), the finite rate of increase (λ) (1.10±0.002 day−1), and net reproductive rates (R_0) (38.60±2.35 offspring per individual) were calculated on okra (Table 5). The longest mean generation time (T) (48.95±2.88 days) and doubling time (DT) (11.55±1.25 days) were documented when *P. solenopsis* was reared on tomato (Table 5).

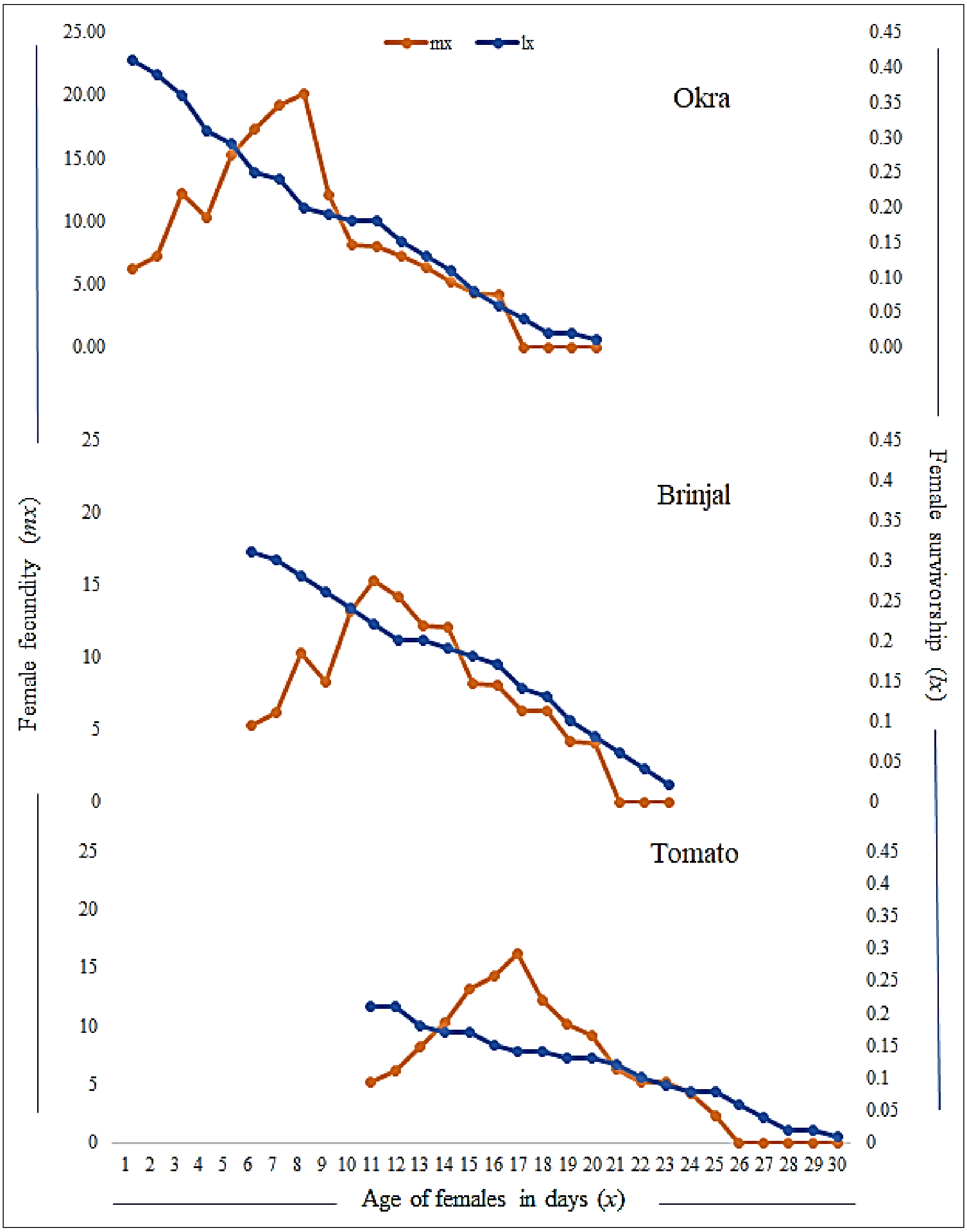


Fig 4: Natality and survivorship of *P. solenopsis* females on different host plants.

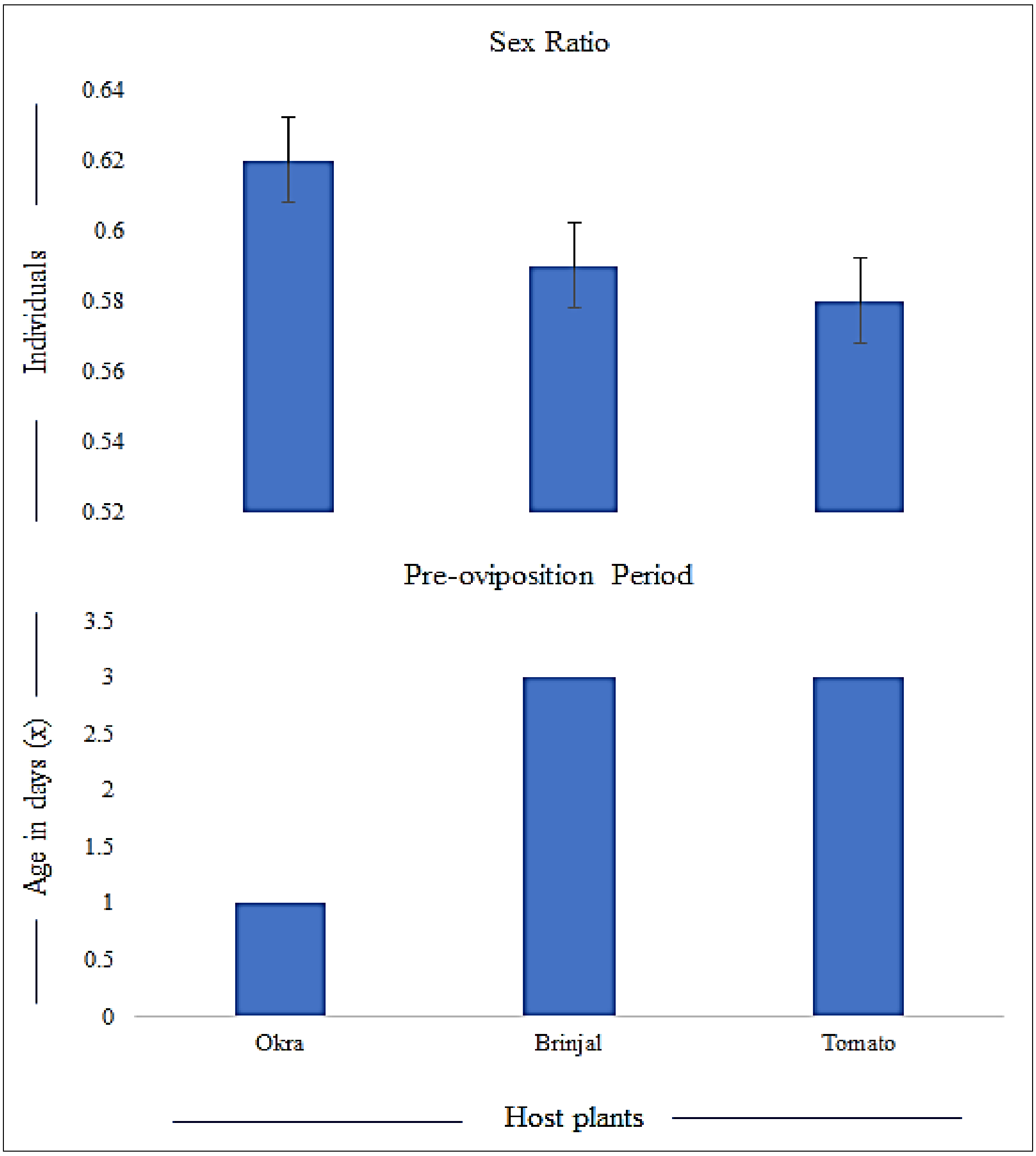


Fig 5: Sex ratio and pre-oviposition period of *P. solenopsis* on different host plants.

Table 3: The life indices of females of *P. solenopsis* on different host plants.

Host plants	pf	R_0	r_m	λ	Tc	τ	DT
Okra	163.33±3.25c	38.60±2.35c	0.09569±0.002c	1.10±0.002c	38.69±1.88a	38.18±1.25a	7.24±1.45a
Brinjal	133.88±2.30c	27.88±2.30b	0.07639±0.002c	1.08±0.001c	43.97±2.25a	43.56±2.25a	9.07±1.15a
Tomato	128.39±1.20c	18.76±1.15c	0.06000±0.001c	1.06±0.002c	48.95±2.88a	48.86±2.26a	11.55±1.25a
df	2,2	2,2	2,2	2,2	2,2	2,2	2,2
F	217.84	222.46	18.54	21.72	327.54	311.16	178.46
P	0	0.023	0	0	0	0	0.015

Means followed by same letter in column are not significantly different ($p<0.05$) by Tukey's HSD

Discussion

Apart from morphology, the plants also differ in terms of metabolites (Wang *et al.*) [41]. These metabolites play a key role in affecting outbreaks, biological and reproductive parameters, and survival rate of a polyphagous pest such as *P. solenopsis*, that combinedly determine the suitability of the host plant for an insect pest (El Aalaoui and Sbaghi) [8]. The cotton mealy bug feeds over 154 species of plants however with varying preference levels (Arif *et al.*) [1].

In present investigation, the outcomes strongly indicate that the selected/tested host plant species significantly affected the development period, reproduction, and overall survival of *P. solenopsis*. Faster development times, lower pre-adult mortality, and higher reproductive rates of insect on a particular host are indicators of host suitability (Saeed *et al.* Dogar *et al.*) [30, 5]. As evident, preference of *P. solenopsis* towards okra may be utilized to manage and mitigate their impact on crops under IPM program (Panizzi and Parra) [27]. According to Panizzi and Parra [27], numerous examples of attractive plants (preferred food sources) are being used today as trap crops in many IPM programs. Often these pest preferred plants are combined with insecticides to bring higher mortality at lower insecticide applications. A plenty of studies are available on host preferences for cotton mealy bug *P. solenospsis* for different host plants *viz.*, Cotton (*Gossypium* spp.), Chinese hibiscus (*Hibiscus rosa-sinensis* L.), potato (*Solanum tuberosum* L.), ornamental plants and weeds, are the most suitable hosts for *P. solenopsis* (Ma *et al.*

Çalískan *et al.* El Aalaoui and Sbaghi. Shahid *et al.* Dogar *et al.*) [44, 4, 8, 33, 5].

It is well proven in the literature that plant nutrients and chemical contents, as well as the phytochemicals produced in response to pest damage bring variations in insect life histories on different hosts plants (Goussain *et al.* Saeed *et al.* Khan *et al.* Tong *et al.*) [12, 30, 18, 36]. The present investigation, the highest peaks of age specific and corresponding stage specific mortality were seen on tomato. Mortality in immature stages of an insect population is a critical factor in assessing the adult population (Saeed *et al.*) [30].

Different host plant species significantly affected the pre-oviposition, oviposition, per day egg laying and egg incubation period of *P. solenopsis*. It is evident from the present findings that okra has been the most suitable host and tomato was the least suitable host plant. Rearing of *P. solenopsis* on okra resulted in significantly longer oviposition period and per day egg laying, and reduced pre-oviposition period over brinjal and tomato. The chemical components of host plants play an important role in the reproductive behaviour of mealy bugs (Reddy and Guerrero. Çalískan *et al.* Dogar *et al.*) [28, 4, 5].

According to Seyfollahi *et al.* [32] and Goundoudaki *et al.* [11] the population growth parameters are the most vital statistical tools to compare the fitness of different plants or the performance of associated insect. The life indices like net reproductive rate, intrinsic growth rate, population doubling time are the key indicators of nutritional and environmental impacts on insect population growth.

In addition, the present outcomes *P. solenopsis* preference towards okra over brinjal and tomato in regarding faster development and higher reproduction may be useful in devising an effective management strategy against this pest, including the determination of time to apply pesticides and release of natural enemies.

Further host preference may better be understood by analysis of chemical composition of the host plants and their impact on anatomical as well as physiological parameters in detail (Panizzi and Parra; El Aalaoui and Sbaghi) [27, 8].

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