Pakistan J. Zool., vol. 50(5), pp 1885-1893, 2018. DOI: http://dx.doi.org/10.17582/journal.pjz/2018.50.5.1885.1893

Effect of Temperature on Demography and Predation Rate of *Menochilus sexmaculatus* (Coleoptera: Coccinellidae) Reared on *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)

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Article Information
Received 22 November 2016
Revised 12 August 2017
Accepted 19 February 2018
Available online 16 August 2018

Authors' Contribution
AI and MAA conceived and designed the study. AI conducted the experiment and analyzed the data. MA provided technical assistance in writing results and discussion. MN and TM guided in research work.

Key words
Age-stage two sex, Life table, *Menochilus sexmaculatus*, *Phenacoccus solenopsis*, Predation rate.

ABSTRACT

Zigzag beetle, *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae) is an important predator of cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Understanding the effect of temperature variations on its demography and predation rate is necessary to predict the population dynamics of this beetle against cotton mealy bug. Age-stage, two sex life tables of zigzag beetle were constructed at three different temperature regimes: 24 ± 0.5 °C and 27 ± 0.5 °C with 60-70% R.H. and ambient condition (32 ± 4 °C; 16-50% R.H.) with 14:10 h (L:D) photoperiod using cotton mealy bug as a host. According to the results, the immature duration and adult longevity were comparatively longer at lower temperature (24 ± 0.5 °C) and shorter at higher temperatures. Among population dynamic parameters, net reproductive rate (R_0) was 216.52, 105.99 and 27.07 off-springs per individual, intrinsic rate of increase (r) was 0.1543, 0.1600 and 0.1518 off-springs per female per day at 27 ± 0.5, 24 ± 0.5, and 32 ± 4.0 °C, respectively. Survival rate (s_{xj}) and age-stage specific fecundity (f_{x7}) were greater at 24 ± 0.5 °C. Among immature stages, 4th instar was the most voracious with highest predation rate. Adult females consumed more cotton mealy bug nymphs at 24 ± 0.5 °C. Net predation rate (C_0) of the beetle was 5548, 4463.2 and 2016.90 at 24 ± 0.5 °C, 27 ± 0.5 °C and 32 ± 4 °C, respectively. The values of transformational rate (Q_p) exhibited that 25.62, 42.11 and 74.50 nymphs of *P. solenopsis* were required per female beetle to lay one egg at 24 ± 0.5 °C, 27 ± 0.5 °C and 32±4°C, respectively. Finite predation rate (ω) was more (7.64) at 24±0.5°C followed by 27±0.5°C (6.56) and the lowest at 32 ± 4 °C (0.66). Adult beetles proved strong natural enemies of *P. solenopsis*. Our study provides detailed basic information for successful rearing of *M. sexmaculatus* in the laboratory and use as a bio-control agent against cotton mealy bug in the field at different temperature conditions.

INTRODUCTION

Cotton mealy bug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) caused losses of 3.1 million cotton bales during 2006-07 in the Punjab, Pakistan (Mahmood *et al*., 2011). It is a polyphagous insect pest which infests almost 194 plant species identified as crops, fruits, vegetables and ornamental plants (Vennila *et al*., 2011; Hameed *et al*., 2012). Ladybird beetles are known to be effective bio-control agents against mealy bugs (Ali and Rizvi, 2009; Michaud, 2001). A large number of predatory coccinellids have been identified as effective bio-control agents of cotton mealy bug (Michaud, 2001; Rafi *et al*., 2005; Ali and Rizvi, 2009). Among these, zigzag beetle *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae) has been suggested to be the promising bio-control agent against cotton mealy bug (Arif *et al*., 2012), as well as other soft bodied insects including aphids, plant hoppers, thrips, jassids, scale insects and white flies (Rahman *et al*., 1993; Solangi *et al*., 2007). This beetle is widely distributed in South Western Asia, Indonesia, Philippines, South Africa, India and Pakistan.

Being poikilothermic organisms, the development, reproduction and predatory potential of insects is affected to a great extent by temperature variations in the environment. Insects perform best only at a certain

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0030-9923/2018/0005-1885 $ 9.00/0

temperature range (Roy *et al*., 2002; Rana, 2006; Pakyari and Enkegaard, 2012). Life table studies of a predator at varying temperatures can provide detailed information about survivorship, development, mortality and life expectancy (Ali and Rizvi, 2007, 2008). Most of the previous studies on *M. sexmaculatus* were focused on exploring its predatory potential against different aphid species (Mari *et al*., 2004; Ali and Rizvi, 2009; Saleem *et al*., 2014) and cotton mealy bug (Arif *et al*., 2011, 2012; Ali *et al*., 2013). An insect with better population growth rate in a particular condition does not necessarily mean an efficient predator (Yu *et al*., 2013).Therefore, predation rate must be considered along with population growth potential in life table under different temperature regimes to assess the efficacy of a particular predator (Chi *et al*., 2011).

The present study was designed to determine the effect of temperature on demographic parameters and predation rate of *M. sexmaculatus* against *P. solenopsis*. This study was based on the hypotheses that (a) temperature influences parameters of demography and predation rate of *M. sexmaculatus* (b) development and predation rates among individuals and between sexes determine the variations of predatory potential of *M. sexmaculatus,* and (c) finite predation rate of (both male and female adult) beetles also influence the predatory potential at a particular temperature.

MATERIALS AND METHODS

Demographic studies of zigzag beetle on cotton mealy bug were conducted at three sets of temperature; 24 ± 0.5 °C, 27 ± 0.5 °C and ambient temperature condition (32 ± 4 °C). In first two temperatures, R. H. was kept at 60-70% and for ambient laboratory condition, the temperature ranged from 28-36°C with 16-50% R. H. First two studies were conducted in growth chamber and the third was conducted in the laboratory at ambient conditions during the months of September and October, 2013. This is actually time of the year when cotton mealy bug population is at its peak in the cotton crop. The ambient temperature condition was used to compare development, reproduction and predation of beetle with those in controlled temperatures to get information for successful mass rearing of *M. sexmaculatus*.

Field collection and rearing

Cotton mealy bug was collected from *Hibiscus rosa-chinensis* Linnaeus (Malvales: Malvaceae) plants and reared on pumpkin *Cucurbita pepo* Linnaeus (Cucurbitales: Cucurbitaceae) fruits in enclosed containers to develop laboratory culture. Adult zigzag beetles were collected from *Parthenium hysterophorus* Linnaeus (Asterales: Asteraceae) plants near University field area and were kept in petri dishes containing cotton mealy bugs and 10% honey solution as a food. Before using for life table studies, twenty pairs of *M. sexmaculatus* were reared on cotton mealy bugs at each set of temperature i.e., 24 °C, 27 °C and ambient condition (32 ± 4°C) for two generations. To maintain genetic variability of predatory zigzag beetles, more number of adult beetles were collected from field and added in the stock culture.

Life table study of Menochilus sexmaculatus

One hundred eggs of *M. sexmaculatus* were shifted to 100 petri dishes each for the three temperature treatments. Moulting and survival of each larval stage from 1st to 4th instar were carefully recorded daily. The adults were paired on the basis of their body size (female larger than male) and transferred to petri-dishes in order to observe their mating behaviour (Mari *et al*., 2004). Survival, adult longevity and fecundity of each female beetle were recorded daily until their death.

We constructed age-stage, two sex life table to estimate life table parameters of the beetles and population dynamics parameters (r, the intrinsic rate of increase; λ finite rate of increase; R_0 the net reproductive rate, T mean generation time) using TWOSEX-MSChart (Chi, 1988, 2012a; Chi and Liu, 1985). Differences in the development time, longevity and reproduction among *M. sexmaculatus* at three different temperatures were analyzed using one way ANOVA followed by multiple comparison with Tukey-Kramer test ($P < 0.05$) (Dunnett, 1980). We used Bootstrap technique to calculate the means and standard errors for population dynamics parameters instead of Jackknife technique to avoid discrepancy between the estimated means (Chi and Yang, 2003).

Predation rate of Menochilus sexmaculatus

Twenty freshly laid eggs were separated from paired culture of *M. sexmaculatus* and shifted to twenty plastic petri dishes individually. After hatching, initially each 1st instar grub was provided with twenty nymphs (1st instar) of cotton mealy bug nymphs as a food. Then, daily consumption of prey increased at different rates as the grubs moulted to next stages under different temperature conditions. Final densities of prey consumed by 4th instars grubs were 280 at 24 ± 0.5 °C, 240 at 27 ± 0.5 °C and 160 at 32 ± 4°C, per day. After emergence from pupae, male and female beetles were paired and provided with different densities (starting from twenty per day) of *P. solenopsis* nymphs, which reached up to 500 at 24 ± 0.5 °C, 440 at 27 ± 0.5 °C and 300 at ambient condition (32 ± 4 °C). Fecundity, survival rate and predation rate of *M. sexmaculatus* were recorded daily till death of individuals.

Table I.- Comparison of developmental duration, adult longevity and fecundity of *M. sexmaculatus* at three different temperature conditions.

Parameters	Temperature					
	24±0.5°C		27±0.5 °C		Ambient condition (32±4 °C)	
	n	Mean±SE	n	Mean±SE	n	Mean±SE
Developmental time (d)						
Egg	86	4.00±0.00a	83	3.00±0.00b	71	2.00±0.00c
Larva 1	76	4.27±0.08a	67	2.87±0.11b	50	1.35± 0.08c
Larva 2	67	3.51±0.06a	57	2.46 ±0.08b	39	1.82±0.08c
Larva 3	60	3.13±0.04a	51	3.33 ±0.06b	34	1.90±0.10c
Larva 4	55	4.68±0.08a	48	4.49±0.07b	33	2.59±0.15c
Pupa	53	4.64±0.07a	45	4.40±0.08b	24	4.00±0.00c
Pre-adult	53	24.3±0.17a	45	20.9 ±0.19b	24	14.2±0.36c
Adult longevity (d)						
Female	38	26.79±0.23a	31	20.52± 0.27b	14	15.07±0.69c
Male	15	17.20±0.26a	14	14.00±0.36b	10	12.80±0.33c
Fecundity (egg/female)	38	570.0±8.99a		342.0 ±7.31b		1936±22.8c
Daily maximum		120		175		92
Lifelong maximum		674		401		315

Temperatures sharing similar letters have no significant difference based on Tukey-Kramer procedure at 5% level of significance.

Data of predation rate of *M. sexmaculatus* individuals at each temperature were analyzed by CONSUME-MSChart (Chi, 2012b). Age-specific predation rate, k_x (mean number of predator beetle to consume prey at age x), age specific net predation rate, q_x (a relationship of age-specific survival rate (l_x) to age-specific predation rate (k_x) which indicates the weighted number of 1st nymphal instar of *P. solenopsis* fed by *M. sexmaculatus* at age x), net predation rate, C_0 (mean number of 1st nymphal instar of *P. solenopsis* consumed by predator beetle during entire life, which is the summation of q_x at all age group of population), transformation rate, Q_p (the ratio of net reproductive rate R_0 over net predation rate C_0 which gives information about the number of *P. solenopsis* consumed by predator beetle for production of eggs), stable predation rate (Ψ) and finite predation rate (ω), were also calculated (Chi *et al*., 2011).

RESULTS AND DISCUSSION

Age-stage two sex, life table of Menochilus sexmaculatus

There was a considerable variation in number of eggs hatching from three cohorts (100 eggs) of *M. sexmaculatus*; 86, 83 and 71 at 24 ± 0.5, 27 ± 0.5 and 32 ± 4 °C, respectively (Table I). Decrease in hatching period with increasing temperature has also been reported by Ali *et al*. (2012) and Veeravel and Baskaran (1996) while studying the predatory potential of *M. sexmaculatus* on aphids. There was a significant decrease in the development periods of larval and pupal stages with increasing temperature. Pre-adult duration was completed in 24.3 days at 24 ± 0.5 °C and in 14.2 days at ambient condition (32 ± 4°C). Same trend was observed in case of adult males and females. Adult female longevity was 26.79 days at 24 ± 0.5 °C and 15.07 days at 32 ± 4 °C, while adult male longevity was recorded 17.20 days at 24 ± 0.5 °C and 12.80 days at 32 ± 4 °C, respectively. Females had longer adult duration as compared to males. Maximal daily fecundity increased from 24 ± 0.5 °C to 27 ± 0.5 °C and decreased at ambient condition. Maximum life time fecundity was the highest (674) at 24 ± 0.5 °C followed by that (401) at 27 ± 0.5 °C and the lowest (315) at ambient condition. Our studies are in agreement with the findings of Mari *et al*. 2004) who reported shorter life span of *M. sexmaculatus* males than females, on alfalfa aphids (*Therioaphis trifolii* Monell). The survival rate and total fecundity of *M. sexmaculatus* was better at 24 ± 0.5 °C and 27 ± 0.5 °C as compared to ambient condition (32 ± 4 °C), which reflected negative effect of high (>28°C) temperature on the performance of *M. sexmaculatus*.

Age-stage specific survival rate (s_{xj}) of *M. sexmaculatus* (the probability that freshly laid eggs will survive to age x and develop to the stage j) was plotted in Figure 1, which showed overlapping curves, illustrating differences in developmental rates in both immature and mature stages. The first adults emerged at the age of 22, 16 and 12 days and the survival rates were 29.08, 50.02 and 17.64 at 24 ± 0.5 °C, 27 ± 0.5 °C and 32 ± 4 °C, respectively (Fig. 1).

The age-specific survival rate (l_x) is the probability of newly hatched egg to survive at age *x*. It ignores the individual developmental rate and stage discrepancy. Age-stage specific fecundity (f_{xj}) explains mean number of eggs laid per adult female at age *x* and stage *j* per day (adult female of *M. sexmaculatus* was at 7th life stage) and only counts female individuals which were able to produce eggs. Age-specific fecundity (m_x) describes the fecundity of emerged larvae to adult age *x*; this is why age-stage specific fecundity (f_{x7}) curves showed higher peaks than the age-specific fecundity (m_x) curves (Fig. 2). First egg laying occurred on 26th, 20th and 15th day with (f_{x7}) value of 0.1944, 1.556 and 1.000 eggs per female per day at 24 ± 0.5 °C, 27 ± 0.5 °C and 32 ± 4 °C, respectively. Age-specific fecundity (m_x) was 0.132, 0.304 and 0.333 females per female per day at age of 26th day, 20th day and 15th day with age specific survival (l_x) was 0.53, 0.46 and 0.24 days at 24 ± 0.5 °C, 27 ± 0.5 °C and 32 ± 4 °C, respectively. The peak of age-stage specific fecundity (f_{x7}) was 71.52, 69.58 and 38.84 eggs per female per day at the age of 33rd, 29th and 20th days, at 24 ± 0.5 °C, 27 ± 0.5 °C and 32 ± 4°C, respectively. Higher peaks of m_x, $l_x m_x$ and f_{x7} were observed at 24 ± 0.5 °C and the lowest at 32 ± 4 °C.

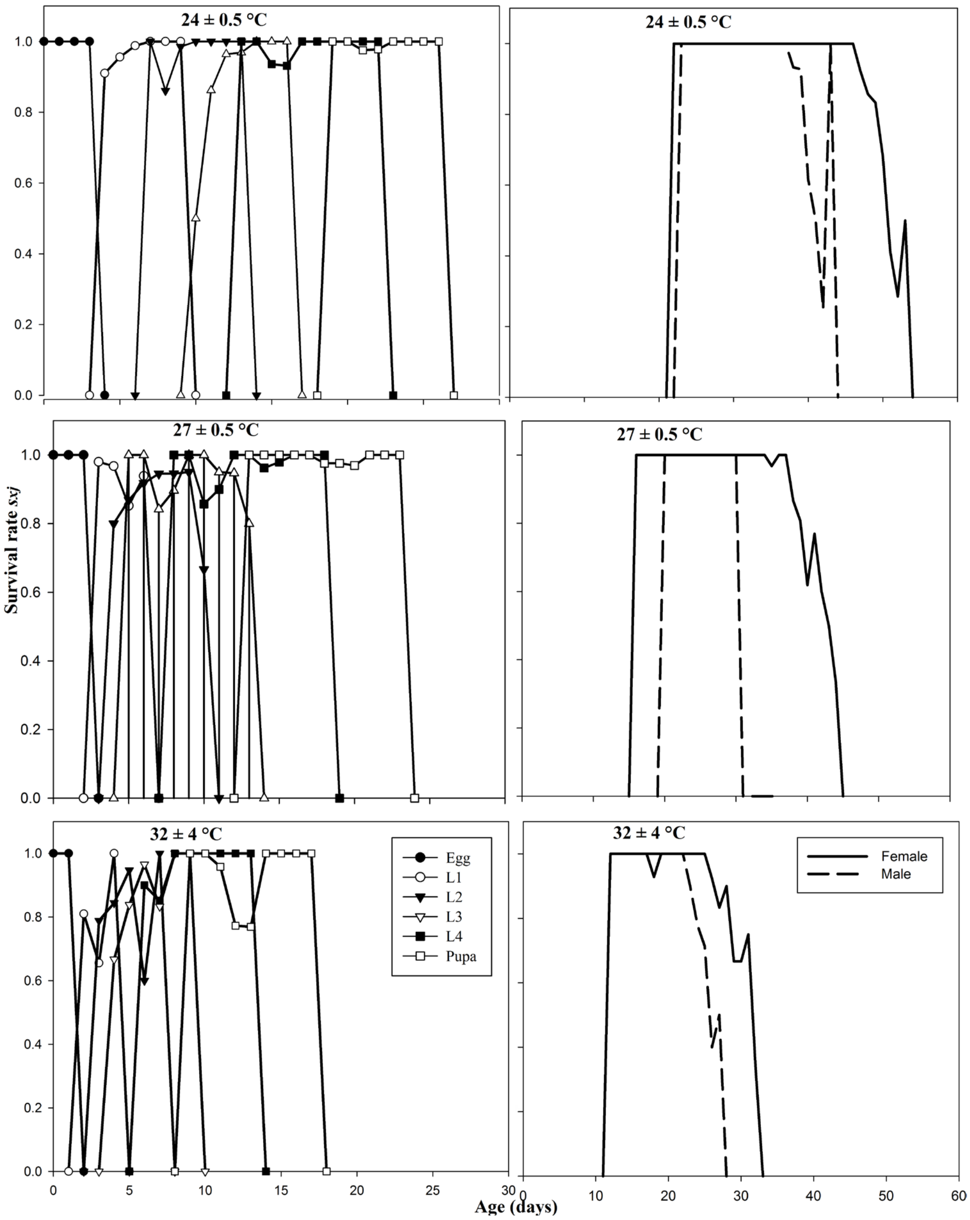

Fig. 1. Age-stage specific survival rate (Sx, i) of *M. sexmaculatus* reared on *P. solenopsis* at different temperatures.

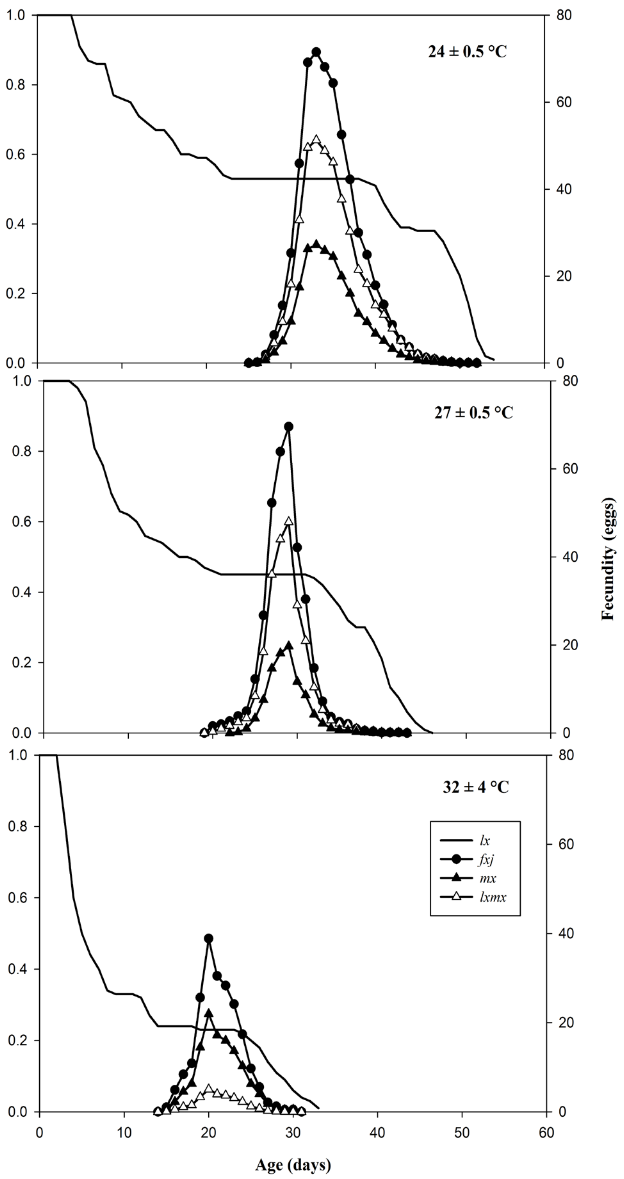

Fig. 2. Age specific survival rates (l_x), fecundities (m_x), maternities ($l_x m_x$) and age-stage specific fecundities (f_{xj}) of *M. sexmaculatus* reared on *P. solenopsis* at different temperatures.

Traditional female age-specific life tables (Birch, 1948) do not provide information regarding stage differentiation, role of male individuals in the population, variable survival rate of different stages and their overlaps in the life history of insects (Chi ,1988; Yu *et al*., 2005; Chi and Su, 2006; Huang and Chi, 2012). We used age-stage, two sex life tables because it accounts variable developmental rate among individuals and stage differentiation of both sexes while calculating population parameters.

Population dynamics parameters

Population dynamic parameters, estimated by using Bootstrap method, reflected strong effect of temperature (Table II) (Efron and Tibshiramin, 1993). Intrinsic rate of increase (*r*) increased with increasing temperature; from 0.1543, 0.1600 and 0.1518 females per female per day at 24 ± 0.5 °C, 27 ± 0.5 °C but declined at 32 ± 4 °C. Due to faster development, higher daily production of eggs and earlier peaks, reproduction was higher at 27 ± 0.5 °C than at 24 ± 0.5 °C. Finite rate of increase (λ) followed the same trend as exhibited by intrinsic rate of increase (*r*). The highest net reproductive rate (R_0) of 216 offspring per individual was noted at 24 ± 0.5 °C and the lowest (27.07 offspring per individual) at 32 ± 4 °C. Mean generation time (*T*) was the longest (34.86 days) at 24 ± 0.5 °C and the shortest (21.73 days) at 32 ± 4°C. Life table studies of *Harmonia dimidiata* on *Aphis gossypii* also showed decrease in mean generation time (*T*) and net reproductive rate (R_0) with increasing temperature Yu *et al* (2013). Insects can tolerate temperature changes only up to certain limits and beyond those limits their life activities are negatively affected (Hameed *et al*., 2012). Same phenomena were observed in the current studies; faster rate of development, higher daily egg production and earlier peaks in reproduction at 27 ± 0.5 °C than at 24 ± 0.5 °C. However, variability in temperature as for ambient condition showed faster development but decreased survival rate and fecundity.

Predation rate of Menochilus sexmaculatus

Predation rate of *M. sexmaculatus* larvae increased rapidly from 1st to 4th instars. Predation increased with the progress of the larval instar (Unal et al., 2017) Total number of mealy bugs fed during immature stages was 1730, 1430.61 and 614 per larva at 24 ± 0.5 °C, 27 ± 0.5 °C and 32 ± 4 °C, respectively (Table III). Predation rate of both female and male adults was higher as compared to different larval instars. During whole life span, female consumed significantly more mealy bugs than those of males at all temperature regimes. *M. sexmaculatus* adult females consumed maximum cotton mealy bugs (5199) at 24 ± 0.5 °C, (3889.7) at 27 ± 0.5 °C and minimum (1905.83) at 32 ± 04 °C. Predation rate of *M. sexmaculatus* increased with age; the fourth instar to be the most voracious among all larval instars. Similar findings were reported by Saleem *et al*. (2014) while studying the predation efficacy of *M. sexmaculatus* against *Macrosiphum rosae* under laboratory conditions. They found 4th instar consuming more preys/day of *Rhopalosiphum maidis, Aphis gossypii and Therioaphis trifolii* than their earlier instars. Female *M. sexmaculatus* with long life period showed more predatory potential than males against *R. padi* at varying temperature conditions Ali *et al*. (2012). In our study, females almost consumed more than twice the number of preys than by

Table II.- Population dynamic parameters of *M. sexmaculatus* reared on *P. solenopsis* at three different temperatures estimated by all individuals and Bootstrap technique.

Parameters		24±0.5 °C	27±0.5 °C	Ambient condition (32±4 °C)
r_m	All individuals	0.1543	0.1600	0.1518
	Bootstrap technique	0.1540±0.0040b	0.1596±0.0057a	0.1497±0.0139c
λ	All individuals	1.1668	1.1735	1.1639
	Bootstrap technique	1.1665±0.0046b	1.1731±0.0066a	1.1616±0.0161c
R_o	All individuals	216.52	105.99	27.07
	Bootstrap technique	216.42±27.93a	105.89±15.81b	26.99±7.36c
T	All individuals	34.86	29.14	21.73
	Bootstrap technique	34.87±0.21a	29.15±0.35b	21.76±0.49c

R_o, net reproductive rate; r_m, intrinsic rate of natural increase; T, mean generation time; λ, finite rate of increase. Temperatures sharing similar letters have no significant difference based on Tukey-Kramer procedure at 5% level of significance.

Table III.- Comparison of predation rate of *M. sexmaculatus* on first nymphal instar of *P. solenopsis* at three different temperatures.

Life stages		24±0.5 °C	27±0.5 °C	Ambient condition (32±4 °C)
Larval stage	L1	98.50±0.95a	51.700±1.140b	14.60±0.82c
	L2	204.0±6.56a	135.45±5.960b	79.60±0.83c
	L3	426.0±10.3a	336.90±13.03b	150.9±1.70c
	L4	996.0±9.28a	899.00±11.16b	371.8±1.98c
Total pre-adult	1st to 4th Instar	1730±10.50a	1430.61±5.55b	614.0±4.39c
Adult stage	Female	5199.0±191a	3889.7±106.1b	1905.83±46.03c
	Male	2349.0±121a	1586.5±93.24b	1282.50±2.020c
Total life span	Female	6947.0±190a	5322.5±110.1b	2521.67±48.43c
	Male	4043.0±138a	3009.50±84.0b	1891.00±1.730c
Net Predation rate (*Co*)		5548.00±436	4463.2±329.34	2016.90±171.25
Transformation rate (*Qp*)		25.62	42.11	74.500
Stable Predation rate (Ψ)		6.550	6.447	0.5632
Finite Predation rate (ω)		7.640	6.5631	0.6559

Temperatures sharing similar letters have no significant difference based on Tukey-Kramer procedure at 5% level of significance.

those by males. This voracity of female may be due to their larger body size and longevity than males, which also require more energy to meet reproduction needs (Farhadi et al., 2011). At ambient conditions, the overall period of all stages was shorter but survival rate and fecundity were lower as compared to those in controlled conditions.

Age-specific net predation, (q_x) is the mean number of cotton mealy bugs consumed by an average individual of *M. sexmaculatus* during its entire life span (Chi and Yang, 2003). Both age-specific predation rate (k_x) and age-specific net predation rate (q_x) showed two obvious curves at egg and pupal stage because at egg and pupal stages do not consume any prey (Fig. 3). These non-predatory phases could not be reflected with traditional life table. This is crucial to decide release intervals of natural enemies for effective biological control program (Yu *et al*., 2013). Net predation rate (C_0) calculated by taking survival rates, predation rates and longevities of the zigzag beetle into consideration was the highest (5548 cotton mealy bugs) at 24 ± 0.5 °C followed by that (4463.2 cotton mealy bugs) at 27 ± 0.5 °C. Transformation rate (Q_p) provides a demographic estimation of the relationship between the reproductive rate and predation rate of the predator (Chi an Yang, 2003). Q_p reflected that *M. sexmaculatus* required 25.62, 42.11 and 74.50 cotton mealy bugs (1st instar) for the production of one egg at 24 ± 0.5 °C, 27 ± 0.5 °C and 32 ± 4 °C, respectively. More preys would be required for the beetle at 24 ± 0.5 °C than at 27 ± 0.5 °C. Contribution of males towards predation of cotton mealy bug was different as compared to females (Table III) and stage differentiation of the beetle showed clear overlaps (Fig. 1). In contrast, traditional life tables theory do not consider sex variation and stage differentiation resulting in overestimation of results regarding predation capacity (Farhadi *et al*., 2011). In the current studies, we calculated both population dynamics parameters and predation rate of *M. sexmaculatus* against cotton mealy bug to avoid such over-estimation.

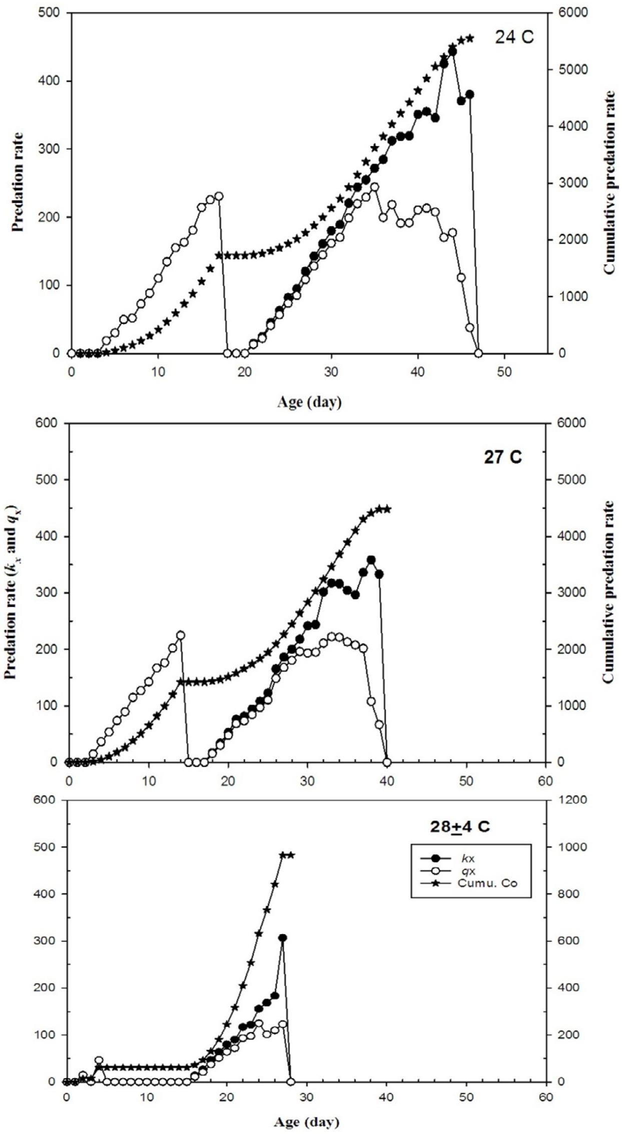

Fig. 3. Age-specific predation rates (*kx*), age-specific net predation rates (*qx*) of *M. sexmaculatus* reared on *P. solenopsis* at different temperatures.

Application of studies in laboratory rearing and bio-control program

Growth potential and efficacy of the rearing program like development rate of larval stage, mortality rate, weight of fresh adults and abundance in the field and life table parameters have been assessed previously (Kalushkov, 1998; Atlehan and Kaydan, 2002; Soroushmehr *et al*., 2008). However, the efficacy of a predator cannot be determined precisely without considering its predation rate along with population growth rate. Although intrinsic rate of increase (*r*) and finite rate of increase (*λ*) of the beetle were higher at 27 ± 0.5 °C, the net predation rate was high at 24 ± 0.5 °C. But higher intrinsic rate of increase (*r*) and finite rate of increase (*λ*) do not indicate higher predatory potential of the predator. Therefore, finite predation rate (*ω*) was calculated keeping into consideration both population growth rate and predation rate to compare the predatory potential of the beetle (Chi *et al*., 2011). Because faster finite predation rate (*ω*) was observed at 24 ± 0.5 °C than 27 ± 0.5 °C, we can consider *M. sexmaculatus* as the more efficient predator of cotton mealy bug at 24 ± 0.5 °C than higher temperature regimes.

CONCLUSION

Stage-specific predation rate and life table parameters were used to assess the predator-prey relationship of *M. sexmaculatus* with *P. solenopsis*. We have demonstrated that, predation rate must be considered with reproductive rate of the beetle during life table studies to explore accurate predation capacity. At 24 ± 0.5 °C, *M. sexmaculatus* is an efficient predator as compared to other temperature regimes. In order to get the next generation earlier, the rearing at 24 ± 0.5 °C will save the labour expenses and consume less prey as a food.

Statement of conflict of interest

Authors have declared no conflict of interest.

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