



## Research Article

# Development and reproductive potential of predatory pirate bug, *Blaptostethus pallescens* (Poppius) at different temperatures

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**ABSTRACT:** *Blaptostethus pallescens* (Poppius) (Hemiptera: Anthocoridae) is a subtropical species and is reported to be a potential predator of sucking pests. Development of this predator was investigated at six different temperatures (15, 20, 25, 30, 35 and 40°C) in the laboratory. Temperature range of 20–30°C was found optimum. Rearing the bug at temperatures lower or higher than this range resulted in the mortality of both adults and pre-imaginal stages. The development of the bug showed a linear relationship with temperature within this range. Maximum fecundity (59 eggs) was recorded at 25 ± 5°C and it was best temperature for mass multiplication of this predatory bug.

**KEY WORDS:** Biology, Biocontrol, *Blaptostethus pallescens* (Poppius), Development, Temperature

(Article chronicle: Received: 24-07-2017; Revised: 10-04-2018; Accepted: 15-05-2018)

## INTRODUCTION

Anthocorids, also known as pirate bugs, belonging to order Hemiptera and family Anthocoridae are one of the many potential predators of sucking pests like thrips, aphids, mealy bugs and mites. In the Mediterranean Basin and sub-Saharan Africa, anthocorids such as *Orius smilis* (Zhang), *O. laevigatus* (Fieber), *O. insidiosus* (Say), *O. majusculus* (Reuter) etc., are considered as important natural enemies in agriculture (Hernandez and Stonedahl, 1999). Pourali *et al.* (2010) and Herard and Chen (1985) reported *Anthocoris minki pistaciae* Wagner and *A. nemorum* (Linnaeus) as another important anthocorid predators against pistachio psylla and pear psylla, respectively. *Blaptostethus pallescens* (Poppius), a subtropical species has been reported to prey on a number of lepidopteran pests and sucking pests such as aphids and spider mites (Tawfik and El-Husseini, 1971).

*Blaptostethus pallescens* was originally described as *B. piceus* Fieber var. *pallescens* (Poppius) from Celebes. Experiments in maize ecosystem of Egypt has shown that it is a potential anthocorid predator of pests (Tawfik and El-Husseini, 1971, Tawfik *et al.*, 1974). In India, *B. kumbi* Rajasekhara had been reported from sugarcane fields in Mysore (Rajasekhara, 1973) and *B. pallescens* from Tamil Nadu, Bombay (Muraleedharan, 1977) and Bangalore (Jalali and Singh, 2002).

Mass multiplication of this bug was initiated on the eggs of *Corcyra cephalonica* (Stainton) in the laboratory at NBAIR, Bangalore and an inexpensive and simple mass

rearing system for this predator was developed (Ballal *et al.*, 2003). Subsequently its predatory potential was established against spider mites and mealy bug on cotton and papaya in India (Ballal *et al.*, 2009, 2012; Kaur *et al.*, 2012; Gupta *et al.*, 2011). Simultaneously Sobhy *et al.*, (2014) conducted experiments to study the biological characteristics of *B. pallescens* so that it can be deployed as augmentative biological control agent in crops such as mango or maize in Egypt. Wide range of prey renders this predator as an ideal candidate for mass rearing and augmentative releases in subtropical areas. However, the existing knowledge for its successful use in biological control programmes of arthropod pests at different agro-climatic regions on different cropping systems in India is scanty with special reference to influence of temperature on development and its basic biological traits and reproductive fitness.

In order to be used as an effective biological control agent the predator needs to be mass reared. Before mass rearing, it is crucial that the role of temperature on development of this potential predator for spider mites is investigated. This is because the basic biological characteristics have been critical for the development of mass rearing protocols for this predator against many sucking pests. Therefore, present study was conducted to determine the effect of temperature on the development and reproduction of *B. pallescens*. Two spotted spider mite, *Tetranychus urticae* Koch has been chosen as a host for this predator in the present study as *T. urticae* assumed a very serious pest on vegetables crops in India during spring, summer and post-

rainy seasons (Gupta, 1985; Singh and Mukherjee, 1991) causing 20 to 45 percent loss in fruit yield (Prasad *et al.*, 2007). Due to failures of chemical control methods, acaricides resistance, harmful effects on non-target organisms etc., the spider mites has been the target of an integrated mite management programs in which potential biological control agents are the key components. These findings will be useful for developing Bio-intensive Integrated Pest Management (BIPM) programs for the management of spider mites in various agro-climatic and cropping systems using this promising biological control agent.

## MATERIAL AND METHODS

### Raising the culture of *Blaptostethus pallescens* in the laboratory

The nucleus culture of *Blaptostethus pallescens* (Accession No. NAN- NBAIR- MP- ANT- 04) was procured from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru India and was maintained according to the standardized method developed by NBAIR.

The nucleus culture of *B. pallescens* nymphs was maintained on deep refrigerated eggs of *Corcyra cephalonica* kept in glass jars (4" × 6") containing thin layer of cotton strands at the bottom covered with a piece of muslin cloth fastened with a rubber band. The prey was provided as and when required until the emergence of adults of *B. pallescens*. The newly formed adults were transferred into ovipositional container (4" × 6") provided with a layer of tissue paper, cotton strands and deep refrigerated eggs of *C. cephalonica* as feed at the base along with three to four pieces of French bean as ovipositional substrate for egg laying. The egg substrates were removed after every 24 hours and replaced with fresh bean pieces for egg laying. The oviposition was confirmed on the substrate (eggs are inserted inside the bean tissues with only operculum visible outside) and shifted to the hatching containers provided with a layer of tissue paper at the base and a thin layer of cotton strands. Eggs of *C. cephalonica* were sprinkled in these jars as a food for newly hatched nymphs and the hatching containers were covered with a piece of black cloth fastened with rubber band. The date and time of oviposition was written on hatching container.

In the hatching containers, red colored nymphs that started hatching within 3–5 days after oviposition were maintained on eggs of *C. cephalonica* as a food until emergence of adults. Sufficient number of different stages of *B. pallescens* was reared for experimental purposes in B.O.D incubator maintained at  $27 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity and L14: D10 photoperiod.

### Raising the culture of *Tetranychus urticae*

The pure culture of two spotted spider mite, *T. urticae* was reared on the potted plants of French beans and brinjal plants under screen house conditions at the Entomology Research Farm, Punjab Agricultural University, Punjab India. In the beginning, females and males of two spotted spider mite were collected from the fields and were released on leaves of French beans kept on moist cotton in petri dishes in the laboratory at room temperature  $30^\circ\text{C}$  and RH 50 per cent. After the completion of one generation on the French bean leaves in the petri plates, the mite bearing leaves were pinned with the help of entomological pins on young leaves of French beans and brinjal plants in the pots for raising culture of mite under screen house conditions. The regular inspection of culture of mite was made to check any infestation of any predator of mite or other insect pest.

### Effect of Temperature on Pre-Imaginal Development Stages of *Blaptostethus pallescens*

Six different constant temperature regimes were tested: 15, 20, 25, 30, 35 and  $40^\circ\text{C}$  to see their influence on pre-imaginal stages. The B.O.D incubators for this experiment were calibrated and constantly maintained within  $\pm 1^\circ\text{C}$  of the test temperature and at  $70 \pm 10\%$  relative humidity and L14: D10 photoperiod. Small sections of French bean pods (5cm size) with newly deposited *B. pallescens* eggs (0–12 hour) were removed from the oviposition jars (stock culture). These were kept in petri dishes (9cm diameter × 1.5cm height) observed daily for egg hatching. Each egg laid on the bean was marked individually. For each temperature the ovipositional substrate was assigned to ten replications with 20 eggs each in a petri dish placed in B.O.D incubator set at six different test temperatures. The eggs were monitored daily for hatching and incubation period was recorded for 200 eggs under each temperature. For recording developmental period, one hundred freshly emerged nymphs were gently removed, assigned to ten replications comprising ten individuals placed in single arena (Petri dishes) for each test temperature as described above. These nymphs were observed daily to record their different stages. During the study, fresh leaves of brinjal infested with *T. urticae* mites were provided to growing nymphs of *B. pallescens* daily till the emergence of adult.

### Effect of temperature on reproduction and adult longevity of *Blaptostethus pallescens*

Pre-oviposition, oviposition and post-oviposition periods along with longevity of adult males and females and progeny production (fecundity) were determined for the adult emerged from the immature stages developed under test temperatures as mentioned in experiment. Adults were

kept at the same temperatures at which they were developed as nymphs (15, 20, 25, 30, 35 and 40 ± 1°C, 70 ± 10% RH, L14: D10 photoperiod). Five newly emerged adults were sexed and placed in small mating jars for 24 hours. After successful mating they were kept in small ovipositional jar provided with layer of tissue paper, cotton strand at the base, brinjal leaf infested with mite (*T. urticae*) as a food and pieces of bean pods for egg laying. There were ten replications comprising five pairs in single arena (jars) placed at six different test temperatures. Females were supplied daily with bean pods as oviposition sites and excess of *T. urticae* as prey until death and longevity was recorded. Bean pods containing deposited eggs were replaced daily and were observed under a stereoscope to determine the number of eggs laid on them.

#### Calculation of development threshold temperature, degree days (°D) and statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using completely randomized design to determine the effect of temperature on developmental and reproductive parameters studied. Post hoc Tukey tests (Tukey's Honest Significant Difference Test) was performed to compare treatment means, if there was significant difference between the treatments indicated by ANOVA. The relationship between temperature and rate of development of pre-imaginal stages of *B. pallescens* was calculated using linear regression.

From linear regression equation ( $y = a + bx$ ; where  $y$  = developmental rate at temperature  $x$ ,  $a$  =  $y$  intercept and  $b$  = slope) of each pre-imaginal stage, lower developmental threshold temperature was calculated for each stage according to the formula:

$$\text{Development threshold temperature} = (y - a)/b \text{ or } (0 - a)/b$$

Where  $y$  was set at zero as attempt was made to determine the temperature at which development stops (Sokal and Rohlf, 1981).

The values of lower developmental threshold were used to calculate the mean degree days as per Wilson and Barnett (1983) formula:

$$\text{Degree days (DD)} = (\text{Threshold temperature} - \text{Particular temperature}) \times (\text{days to develop at particular temperature})$$

The lower developmental threshold for an organism is the temperature below which the development stops. The total amount of heat required between lower and up-

per threshold for an organism to develop from one point to another in life cycle is calculated in units called degree days (°D). Degree days are accumulated product of time and temperature threshold for each day. Development can be estimated by accumulating degree days between temperature thresholds ([www.ipm.ucdavis.edu/weather/ddconcepts](http://www.ipm.ucdavis.edu/weather/ddconcepts)).

## RESULTS AND DISCUSSION

### Effect of temperature on Pre-Imaginal developmental stages of *Blaptostethus pallescens*

Significant differences were found in developmental period for all stages of *B. pallescens* nymphs under the tested temperature (15, 20, 25, 30, 35 and 40°C). In general time taken to complete the development by all the nymphal stages was shortened with increase in temperature. However, a high rate of mortality of eggs and nymphs was observed at temperature lower (15°C) or above 30°C. Despite of repeated observations huge mortality was observed. So these temperature regimes (≤15°C and above 30°C) were not considered for further studies.

The incubation period of eggs to hatch was significantly short at 30°C (3.20 ± 0.22 days), but it was prolonged to 4.80 ± 0.25 days and 9.40 ± 0.48 days when the eggs were kept at lower temperatures of 25°C and 20°C, respectively. All the nymphal stages of *B. pallescens* i.e., 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> seemed to develop very slowly at lower temperature of 20°C whereas they took lesser days at 30°C to complete their respective developmental stage, which resulted in longer nymphal period at 20°C (33.36 ± 0.09 days) and shorter nymphal period at 30°C (14.49 ± 0.09 days) (Table 1). Therefore, the overall development (from eggs to adults) of *B. pallescens* was observed to be faster at higher temperature (30°C) as compared to lower temperature (20°C) i.e., *B. pallescens* took fewer days at 30°C (17.69 ± 0.31 days) as compared to 20°C (42.76 ± 0.50 days) and 25°C (28.23 ± 0.17 days) to complete the development. The present data also showed that the development of *B. pallescens* was relatively linear within 20–30°C (Fig. 1). The time taken to complete the development period by all pre-imaginal stages of *B. pallescens* decreased with increase in temperature from 20 to 30°C. The insects are cold blooded organisms and the temperature has great influence on their development rate. The present results show that development rate of *B. pallescens* increases as the temperature increases. In the range of 20–30°C, the development rate changes almost linearly with increase in temperature. At very low temperature (15°C) and very high temperature (above 30°C), there is no development of predator. These findings corroborate the results of Sobhy *et al* (2014), who reported development time

of *B. pallescens* to be faster as the temperature increases. According to them, the development of *B. pallescens* was 1.5 folds faster at 25°C than 20°C and it was again 1.5 folds faster at 30°C as compared to 25°C. Similarly, Pourali *et*

*al.* (2010) showed that *A. minki pistaciae*, a closely related predatory bug against pistachio psylla, developed successfully within a wide range of temperatures (17.5 to 32°C).

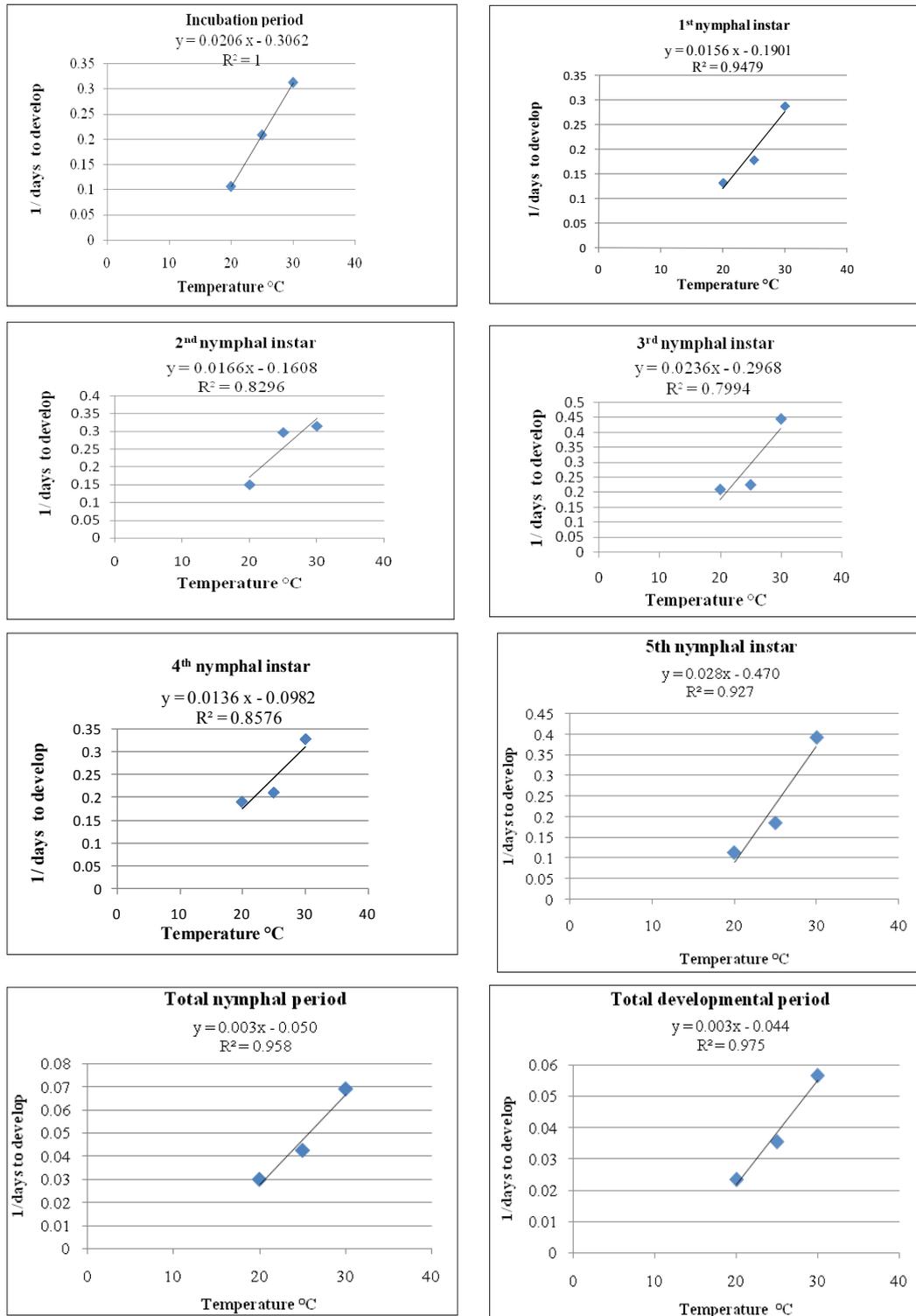


Fig. 1. Relationship between temperature and rate of development of *Blaptostethus pallescens*.

In the present studies, it is shown that the lower development threshold temperature from eggs to adult emergence varied between 7.00 and 16.79°C with a mean Degree Day (DD) requirement between 24.38 and 299.6°D (Table 2). Since temperature influences growth and development of insect, each developmental stage of insect is dependent on temperature and certain amount of heat to complete development process. According to Wagner *et al.* (1984) as the insect approaches its thermal maximum, the changes in the rate of development slowed down. In the present studies, each developmental stage of *B. pallescens* required particular lower development threshold temperature and heat (DD) to complete the development as shown in table 2. The development of each pre-imaginal stage of *B. pallescens* slowed down or nymphs died below 20°C or above 30°C. Therefore, the data of lower temperature threshold for *B. pallescens* in the present studies shows that below this temperature there is no development of *B. pallescens* and optimum range of temperature for the normal development of *B. pallescens* came to be 20 to 30°C.

Hart *et al.* (1997) estimated that the development threshold temperature from oviposition to adult emergence varied between 6.0°C and 7.1°C with a degree day requirement between 256 and 280°D for *Episyrphus balteatus*, a predator of aphids in UK and this data provided useful basis to develop thermal model for the potential number of generations of *E. balteatus* in UK. According to Chhagan and Stevens (2007) 419.8 degree days, above lower threshold temperature of 10.1°C, were required to complete development of green house thrips (*Heliothrips haemorrhoidalis* Bouché) from egg to adult on lemon fruit and they proposed the information valuable in determining the optimum time for initiating greenhouse thrips monitoring programme or for applying control measures against thrips. Padmavathi *et al.*, (2013) estimated 11.1°C lower threshold temperature and thermal constant of 455 degree days for rice leaf folder, *Cnaphalocrocis medinalis* Guenée and suggested the involvement of enzymatic activity above lower threshold temperature to complete development process.

**Table 1. Influence of temperature on pre-imaginal stages of *Blaptostethus pallescens***

Developmental stage of <i>B. pallescens</i>	Duration period (days) at different temperatures (C) (Mean ± SE)		
	20	25	30
Incubation Period (F = 88.009; df = 2; p = 0.000)	9.40 ± 0.48 <sup>a</sup>	4.80 ± 0.25 <sup>b</sup>	3.20 ± 0.22 <sup>c</sup>
1 <sup>st</sup> Nymphal Instar (F = 3274.774; df = 2; p = 0.000)	7.56 ± 0.05 <sup>a</sup>	5.60 ± 0.03 <sup>b</sup>	3.47 ± 0.03 <sup>c</sup>
2 <sup>nd</sup> Nymphal Instar (F = 1151.739; df = 2; p = 0.000)	6.96 ± 0.05 <sup>a</sup>	3.36 ± 0.08 <sup>b</sup>	3.17 ± 0.52 <sup>b</sup>
3 <sup>rd</sup> Nymphal Instar (F = 1025.027; df = 2; p = 0.000)	4.76 ± 0.04 <sup>a</sup>	4.43 ± 0.003 <sup>b</sup>	2.24 ± 0.04 <sup>c</sup>
4 <sup>th</sup> Nymphal Instar (F = 70.083; df = 2; p = 0.000)	5.25 ± 0.05 <sup>a</sup>	4.75 ± 0.22 <sup>b</sup>	3.06 ± 0.06 <sup>c</sup>
5 <sup>th</sup> Nymphal Instar (F = 784.537; df = 2; p = 0.000)	8.83 ± 0.02 <sup>a</sup>	5.39 ± 0.16 <sup>b</sup>	2.54 ± 0.80 <sup>c</sup>
Total Nymphal Period (F = 3959.373; df = 2; p = 0.000)	33.36 ± 0.09 <sup>a</sup>	23.53 ± 0.22 <sup>b</sup>	14.49 ± 0.09 <sup>c</sup>
Total Developmental Period (F = 1254.098; df = 2; p = 0.000)	42.76 ± 0.50 <sup>a</sup>	28.23 ± 0.17 <sup>b</sup>	17.69 ± 0.31 <sup>c</sup>

Comparison has been done temperature wise for different parameters. Values followed with different superscripts are significantly different using Tukey's test (p < 0.05)

**Table 2. Developmental threshold temperature and Degree days (°D) for pre-imaginal stages of *Blaptostethus pallescens* at different temperatures**

Life stages	Developmental threshold temperature (°C)	*Degree days accumulated at temperature (°C)			Mean degree days (°D)
		20°C	25°C	30°C	
Incubation period	14.8	48.88	48.96	48.64	48.82
1 <sup>st</sup> nymphal instar	12.18	59.11	71.79	61.8	64.23

2 <sup>nd</sup> nymphal instar	9.68	69.04	51.47	64.41	61.64
3 <sup>rd</sup> nymphal instar	12.57	35.36	55.06	39.04	43.14
4 <sup>th</sup> nymphal instar	7.22	67.09	84.45	69.70	73.74
5 <sup>th</sup> nymphal instar	16.79	28.34	44.25	33.55	24.38
Total nymphal period	12.92	236.18	284.24	247.48	255.96
Total developmental period	13.45	280.08	326.05	292.78	299.64

\*Calculated on the basis of developmental threshold temperature using Wilson and Barnett (1983) formulae

### Effect of temperature on reproductive phases and adult longevity of *Blaptostethus pallescens*

The individual performance of adult female in terms of total fecundity was significantly higher at 25°C (59.0 ± 1.58 eggs/female) and lowest at 30°C (41.6 ± 2.67 eggs/female), which did not differentiated significantly at 20°C (41.8 ± 1.51 eggs/female). In case of daily fecundity, it was higher at 30°C (2.97 ± 0.19 eggs/female), as well as at 25°C (2.70 ± 0.07 eggs/female) and comparatively lower at 20°C (1.39 ± 0.05 eggs/female). In the present study, the pre-oviposition, oviposition periods and post-oviposition period were significantly different at all three temperatures (20, 25 and 30°), being shorter at 30°C (2.58 ± 0.05 days, 12.40 ± 0.54 days and 2.87 ± 0.07 days, respectively) followed by 25°C (4.20 ± 0.31 days, 20.20 ± 0.47 days and 4.37 ± 0.13 days, respectively) and longer at 20°C (8.73 ± 0.05 days, 28.00 ± 0.54 days and 5.23 ± 0.08 days, respectively) (Table 3).

There was significant difference on the life span of male and female adults which survived at all three temperatures i.e., 20, 25 and 30°C. Male and female adults survived for longer period of time at 20°C (39.10 ± 0.52 and 53.25 ± 0.50 days, respectively), however when studied at 25 and 30°C there was no significant difference in the longevity of males but there was significant difference in case of longevity of females at 25°C (32.47 ± 0.67 days) and 30°C (24.84 ± 0.18 days) (Table 3).

The results of reproduction in the present studies were in accordance with the findings of Sobhy *et al.*, (2014), who observed highest fecundity at 25°C (84.25 eggs) but a decrease by 25 per cent (65.15 eggs/female) at 30°C. However, the daily fecundity was higher at 30°C when *B. pallescens* was studied at different temperatures and fed on *Ephestia kuehniella* (Zeller) eggs. Sobhy *et al.*, (2014) assessed the longevity of adults of *B. pallescens* fed on the eggs of *E. kuehniella*, which appeared to be decreased with

**Table 3. Effect of temperature on reproductive phases and adult longevity of *Blaptostethus pallescens***

Reproductive phases/ adult longevity	Temperature (°C) (Mean ± SE)		
	20	25	30
Pre-oviposition period (days) (F = 1308.322; df = 2; p = 0.000)	8.73 ± 0.05 <sup>a</sup>	4.20 ± 0.31 <sup>b</sup>	2.58 ± 0.05 <sup>c</sup>
Oviposition period (days) (F = 228.150; df = 2; p = 0.000)	28.00 ± 0.54 <sup>a</sup>	20.20 ± 0.47 <sup>b</sup>	12.40 ± 0.54 <sup>c</sup>
Post-Oviposition Period (Days) (F = 150.279; df = 2; p = 0.000)	5.23 ± 0.08 <sup>a</sup>	4.37 ± 0.13 <sup>a</sup>	2.87 ± 0.07 <sup>c</sup>
Fecundity/ day/ female (number of eggs) (F = 46.868; df = 2; p = 0.000)	1.39 ± 0.05 <sup>b</sup>	2.70 ± 0.07 <sup>a</sup>	2.97 ± 0.19 <sup>a</sup>
Total fecundity/female (number of eggs) (F = 25.083; df = 2; p = 0.000)	41.80 ± 1.51 <sup>a</sup>	59.0 ± 1.58 <sup>b</sup>	41.6 ± 2.67 <sup>b</sup>
Male longevity (days) (F = 103.883; df = 2; p = 0.000)	39.10 ± 0.52 <sup>a</sup>	20.98 ± 0.20 <sup>b</sup>	16.67 ± 0.13 <sup>c</sup>
Female longevity (days) (F = 874.210; df = 2; p = 0.000)	53.25 ± 0.50 <sup>a</sup>	32.74 ± 0.67 <sup>b</sup>	24.84 ± 0.18 <sup>c</sup>

Comparison has been done temperature wise for different parameters. Values followed with different superscripts are significantly different using Tukey's test (p < 0.05)

increasing temperature from 20 to 30°C. There was an increase in reproductive success (fecundity) and decrease in preoviposition, oviposition and post-oviposition period of *B. pallescens* with increase in temperature from 20 to 30°C in the present studies which are in agreement with the studies of Sobhy *et al.*, (2014), which found same temperature dependent effect on reproductive success of *B. pallescens* reared on the eggs of *E. kuehniella*. The decline in temperature might delay the oocyte development and cause a prolongation of pre-oviposition period as observed in the present case. Further, the females of *B. pallescens* laid maximum number of eggs (total fecundity) at 25°C (59.0 eggs) as compared to 30°C (41.8 eggs).

The pre-oviposition period of females reared at 25°C was significantly longer (4.20 days) than those reared at 30°C (2.58 days). Therefore, females reared at either temperature could begin laying eggs at similar times and lay similar number of eggs each day. However, the greater longevity of females reared at 25°C would enable them to produce more offspring. In fact, lifetime fecundity was 1.4 times higher at 25°C than at 30°C. Studies of Ahmadi *et al.*, (2007) on *Orius similis* (Zhang), which is closely related to *B. pallescens*, also supported the present findings i.e., there was an inverse relationship between longevity and temperature.

Based on the present data on the ability of *B. pallescens* to develop and reproduce at different temperatures, temperature in the range of 20–30°C appeared to be well within its development thresholds. It indicated that being sub-tropical species, the pre-imaginal development of *B. pallescens* might be temperature dependent, either the development or reproduction being significantly faster at 30°C and 25°C, respectively than at lower temperature (20°C or below). The present studies could be helpful in developing releasing technology of *B. pallescens* for the management of spider mite under different climatic conditions.

#### ACKNOWLEDGEMENT

The authors are grateful to Head, Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab for providing infrastructure and necessary facilities during the course of study.

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