



E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(5): 1016-1022
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Received: 17-07-2016
Accepted: 18-08-2016

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Effect of temperature on biological attributes and predatory potential of *Harmonia dimidiata* (Fab.) (Coleoptera: Coccinellidae) fed on *Rhopalosiphum padi* aphid

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Abstract

The influence of temperature on the biology and prey consumption potential of ladybird beetle, *Harmonia dimidiata* (Fab.) (Coleoptera: Coccinellidae), fed on *Rhopalosiphum padi* aphid was investigated. The results showed that temperature had significant effect on developmental duration, survival and prey consumption potential of different stages. The duration of different stages was highest at low temperature and shortest at high temperature levels. Although the predator completed its development at all tested temperatures but extreme low and high temperature adversely affected the survival of immature stages. At high temperature level, the female beetle could not produce eggs. The lower temperature threshold level for different stages were in the range of (-1.25 °C to - 0 °C) and thermal constant k values for different stages were in the range of (17.86 °D to 142.86 °D). The results indicate that optimum temperature for rearing *H. dimidiata* was 24 ± 1 °C. The predatory ladybird beetle can be utilized as a part of integrated pest management program for the management aphid pests.

Keywords: *Harmonia dimidiata*, *Rhopalosiphum padi*, temperature, biology, predatory potential

Introduction

Lady bird beetles are considered as the most important natural enemies of aphids and have been effectively utilized for the integrated control of several aphid pests. Ladybird beetles are generalist predators that fed on a diverse range of insects [1]. Aphids are the principal prey of ladybirds, whereas coccids, mites, honeydew, pollen, nectar and mildew are considered as secondary foods [2]. There are about 4400 predatory Coccinellid beetles in the world and their potential as bio control agents for different pests is quite promising. The sub-tropical ladybird beetle *H. dimidiata* (Fab.), commonly known as fifteen spotted lady bird occurs in China, Vietnam, India, Nepal, Pakistan and other countries of South-East Asia. In Pakistan it was reported from Malakand, Swat, Murree, Peshawar, Islamabad and Rawlakot [3]. Both larvae and adult ladybird beetle preys on different aphid species such as *Myzus persicae*, *Schizaphis graminum*, *Aphis gossypii*, *Aphis fabae* and *Acyrtosiphon pisum* [4].

H. dimidiata is highly voracious predator of aphids and has the ability to survive even at low temperatures [4]. These attributes make it a useful natural enemy for the purpose of biological control program against aphid pests of economically importance in sub-tropical regions of Pakistan. [14]. Among abiotic factors, temperature is the most important ecological aspect that strongly influences the rate of growth in insects and thereby affecting their seasonal population [5]. To evaluate the prospective of an insect as bio control agent, it is imperative to recognize its temperature necessities in relation to prey, such as low and high temperature thresholds as well as optimal temperature calculated for major life characteristics of insect. Increase in temperature usually increase the metabolic demand, as a result, predation and insect development rate tend to increase exponentially [6]. The effect of temperature is usually determined as a specific function of temperature and its effect on survival, fecundity, demographic parameters, growth rate and development, which is then used for predicting interaction among bio control agents and their host insects [7]. Temperature driven models are often used to estimate the seasonal population dynamics of pests and natural enemies in field situations [8]. These models also assist to conclude apposite circumstances for mass rearing of natural enemies [9].

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Degree-day models, based on linear regressions of development rate and temperature, are used extensively for foreseeing insect development within a variety of temperatures, characteristically met in the field [10]. The temperature below which no quantifiable development of any species takes place is called as lower threshold temperature for development. The amount of heat required over time for an insect to complete the development is considered to be thermal constant. Insect faces a variety of temperatures, which seriously distress their development and survival rate. The study of relationship of temperature with development of notorious pests is very important. The effect of temperature is usually observed in insect species functions, survival of different stages, female fecundity, demographic parameters, and development, which are applied to envisage interactions of natural enemy with pests [11].

Though detailed knowledge of the temperature dependent biology of *H. dimidiata* has been little studied in the world and particularly in Pakistan no such study available in past, therefore the present laboratory experiments were undertaken to study the effect of five constant temperatures on survival, biological parameters and prey consumption potential of larvae and adult beetles to find out the thermal requirements. This study should be useful in predicting the range of temperature within which *H. dimidiata* shows a linear developmental response and thus will help in selecting the appropriate temperature for the laboratory rearing to explore their potential as bio control agents against aphid pests of economic importance.

Material and methods

Study was carried out to check the effect of five constant temperatures i.e. 16, 20, 24, 28 and 32°C with variation of $\pm 1^\circ\text{C}$ under $60 \pm 5\%$ relative humidity and (16: 8) hours (Light: Dark) photoperiods on the biological attributes and predatory potential of ladybird beetle *H. dimidiata*, fed on *R. padi* aphid in growth chamber at National Agricultural Research Centre, Islamabad during 2012.

The following sets of experiments were conducted.

Effect of temperature on biological parameters and percent survival of *H. dimidiata*

A total of 100 eggs of *H. dimidiata* were collected from the stock culture maintained on *R. padi* aphid in laboratory for each treatment. The egg batches were kept in petri dishes for hatching. Upon hatching the larvae were individually placed in plastic vials (6 x 3) cm. Aphids were provided on wheat leaves inside the vials daily. After each 24 hours the old infested leaves were replaced with new diet. The insect were observed for survival and moulting daily. The insect passes through four larval instars. The exuviae found in the vial was the indication that the insect entered into next instar. The procedure was followed till all the larvae entered into pupal stage. Upon adult emergence from pupae at temperature level, paired male and female beetles were shifted to rearing jars (4"x12"), lined with filter paper to facilitate egg laying. The jars were kept under the same temperature. Mixed stages of aphid's (i-iv) nymphal instars were provided as food inside the rearing jars on wheat leaves in excess daily. After each 24 hours the old infested leaves were replaced with new diet in each jar. The insects were observed daily twice for egg laying. The first egg observed in each pair was the indication for the end of pre oviposition period. The eggs laid on wheat leaves or on filter paper inside the jars in masses were collected daily

throughout the oviposition period of female ladybird beetles. The observations were carried out till all the male and female beetles were died in each jar at all tested temperatures. The longevity of both sexes was recorded. During the female longevity experiment, the first and last egg was recorded in each pairs to separate, pre oviposition, oviposition and post oviposition periods.

The data was recorded on the following parameters.

1. Incubation period (days) and hatchability of eggs
2. Developmental period and survival of different larval instars/larvae
3. Pre Pupal and pupal durations and percent survival
4. Total developmental period and percent survival from egg to adult emergence
5. Sex ratio, number of male and female beetles emerged
6. Pre oviposition, oviposition and post oviposition period of female beetle
7. Longevity of male and female ladybird beetles
8. Mean number of eggs per female beetles during their life span
9. Mean number of eggs per female per day

Temperature thresholds and thermal requirements for developmental stages of *H. dimidiata* (Fab.)

Temperature threshold and thermal prerequisite were calculated by using degree day model to approximate the linear association of temperature and the developmental duration of *H. dimidiata* fed on *R. padi* aphid. The reciprocal of developmental period for egg, larvae, pupa, egg to adult emergence, male and female beetles was computed to attain the rate of development (1/day) at all tested temperatures. Linear regression lines were used to calculate the association among growth rate and temperature and to find out intercept (a) and slope (b). With the help of liner regression lines the lower threshold temperature (T_0) for each developmental stage was calculated. Once the lower temperature threshold for each developmental stage was attained then the thermal constant K (the number of degree days required to complete development for each stage) was calculated from the reciprocals of the fitted regression line (b^{-1}). Thus the degree days required for the development of each life stage were estimated.

Effect of temperature on the predatory potential of *H. dimidiata* larvae and adult beetles

To study the effect of five constant temperatures on predatory potential of larvae, a total of 40 first instar larvae were kept in rearing vials singly at each temperature in growth chamber. Counted numbers of aphids were provided as food on wheat leaves inside the vials daily. The first and second instar larvae were provided (30-50) first and second instar nymphs of aphids on wheat leaves. As the age of the larvae increased, third and fourth instar nymphs of aphids were provided as food to third and fourth instar larvae of predator. The numbers of aphids as food was increased subsequently up to 150 aphids/ day to the fourth instar larvae. The diet was replaced after each 24 hours with fresh diet. The number of consumed and unconsumed aphids was recorded daily. Whenever the larvae were found dead in the vials, they were replaced with new larvae of the same age. Therefore each larval stage was replicated 40 times at each temperature level. The larvae were fed till all the larvae entered in to pre pupal and subsequently to pupal stage.

Similarly to study the effect of temperatures on predatory potential of adult's ladybird beetles a total of 20 freshly

emerged male and female beetles were taken from established culture of ladybird beetles. The male and female beetles were set aside separately in rearing jars (4"x12") at five constant temperatures in growth chamber. Counted numbers of aphid approximately (250-300) mixed stages usually (i-iv) nymphal instars of aphids were provided inside the jars on infested wheat leaves as food daily. After each 24 hours, the consumed and unconsumed aphids were counted in each rearing jars and the old infested leaves were changed and fresh leaves with counted aphids were provided. The process was continued till all male and female ladybird beetles were found dead at all tested temperatures.

The data was recorded on the following parameters

1. Mean number of aphids consumed during their life span and mean number of aphids consumed/day by first, second, third and fourth instars larvae
2. Mean number of total aphids consumed per larvae during their life span and mean number of aphids consumed/day
3. Mean total number of aphids eaten per adult male and female beetles during their life span
4. Mean number of aphids consumed per adult male and female beetles/ day

Data Analysis

The data on the developmental duration of different stages was subjected to one way ANOVA using Statistix 8.1 package. Means were compared using Turkey (HSD) test at 5% level of significance (unequal replicates for each stage). While the data on the predatory potential of larvae and adult male and female ladybird beetle were also subjected to one way ANOVA and the means were compared using (LSD) test at 5% level of significance (equal replicates for each stage).

Microsoft excel program was used for making linear regression lines. The lower threshold temperatures (T_0) and thermal constants (K) were establish by drawing linear regression lines between developmental rates (1 / days) and temperatures. The (T_0) and (K) were calculated from the linear regression equation $y = a + bx$, where y = rate of development and x = temperature) and $T_0 = -a / b$, and $K = 1 / b$ [10].

Results and discussion

Temperature showed profound effect on the developmental durations and survival rate of immature stages of *H. dimidiata*. The durations of different developmental stages significantly decreased with increasing temperature. The incubation period was maximum (9.06±0.07) days at low temperature 16 ±1 °C and minimum (3.02±0.08) days at high temperature level 32±1 °C (Table 1). The incubation period was considerably different at all tested temperatures except at higher temperatures levels 28±1°C and 32±1°C. The results indicate that temperature had a significant effect on the incubation period. The same trend was found for the duration of different larval instars, larvae, pre pupal and pupal stages, where the duration was longest at low temperature and shortest at high temperature levels (Table 1). The total duration from egg to adult emergence was longest (50.87 ±0.75) days at low temperature level and shortest (17.62±0.23) days at high temperature level.

Yu *et al.* (2013) reported (38.8 ±0.3, 27.5 ±0.1 and 18.4 ±0.1) days at 15, 20 and 25°C respectively. While Kuznetsov and Pong (2002) reported (35 and 22) days duration of immature stages and the larvae duration was (22 and 12) days at 20°C and 25 °C. The present results are similar to Veeravel and Baskaran (1996), who used four temperatures in their studies

and observed the effect of temperature on growth, development, fecundity, prey consumption capability and adult durations of two Coccinellid beetles *Coccinella transversalis* (Fab.) and *Menochilus sexmaculatus* (Fab.). Increase in temperature resulted in fast development of both Coccinellid beetles, which results in reduction of developmental and ovipositional periods. The oviposition period was longest at lower temperature 20 °C. The duration of adult ladybird beetles and total life span decreased with increased in temperature. In the present study the durations of pre adult stages exhibit a temperature dependent trend. Considering the duration of the pre-adult stages, the data obtained revealed that all the developmental stages were significantly affected by temperature. In the course of the present study temperature has shown profound effect on the adult biology and as well as reproductive potential of female beetle. At extreme high temperature (32 ±1 °C), the female beetle could not produce eggs. The present results are in close conformity with Yu *et al.* (2013), who also reported that female *H. dimidiata* could not reproduce at higher temperature (30 °C). In the present study eggs per female beetle were highest at 24 ±1 °C, followed by 20 ±1 °C. While extreme low and high temperatures have deteriorated effect on fecundity of female beetle. Previous workers, W. A Gillani *et al.*, (2007) and Semyanov (1999) also reported different number of eggs per female, when fed on different aphid species. These differences probably may be due to different host insects they used for feeding or may be due to inherited differences in populations with other geographical origin and strain of *H. dimidiata* they used for experiments.

The survival rate from egg to adult emergence was also different from each other. Maximum survival of immature stages was (78.0%) at 24 ±1 °C followed by 20±1 °C and minimum survival was only (15%) at highest temperature level 32± 1 °C. The results indicate that the most favorable temperature was 24 ±1 °C. The results of the present study are also in conformity with [16] who reported that the most favorable temperature range for rearing of *H. dimidiata* was in the range of 20 to 25 °C

Assessment of lower threshold temperatures and thermal constants from linear regressions for different stages, responded to temperature in a similar way. It was assumed that *H. dimidiata* could tolerate the range of temperature from 16 °C to 32 °C appropriate to its distribution. However, the results of the present study shows that temperature (32 ±1 °C) is above the optimum temperature range for some aspects of *H. dimidiata* life attributes. At this temperature, female could not produce eggs and high mortality was observed particularly during egg and first instar larval stage and total emergence from egg to adult stage was also minimum. The most appropriate rate for development is between the ranges of 16 ±1 °C to 28 ±1 °C. The lower threshold and thermal constant k (degree days) was calculated for different stages. The thermal constant for egg, larvae, pupae and egg to adult emergence was (-1.25 °C, -0.86 °C, -0.96 °C and -1 °C. The thermal constant for adult male and female beetles (- 0 °C). Similarly the degree day for egg, larvae, pupae, egg to adult emergence and adult male, female was 17.86 °D, 66.6 °D, 20.41 °D, 11.1 °D and 142.86 °D, 123.46 °D respectively (Table 3). Although comprehensive work has been done in the past on the development threshold temperature and effective accumulated temperature degree day on Coccinellid beetles, but according to available literature no such type study was conducted on *H. dimidiata* in the past. Chen XF (2000) reported that the lower threshold temperature was 11.9 °C and

degree day was 452.3 DD for *C. montrouzieri* beetle. According to Atlihan R *et al.*, (2008), the thermal summation of Coccinellid beetle *Scymnus subvillosus* (Gorze) was 77.5, 145.8 and 300 DD for egg, larval and total pre-adult stages. Stathas GJ (2011) recorded the lower developmental threshold and thermal summation of ladybird beetle *H. axyridis* (Pallas) for egg, total pre-imaginal time and life cycle as 10.2 °C and 47.8 DD, 11.17 °C and 258.3 DD and 11.8 °C and 357.1 DD when reared on *A. fabae* (S.). Hodek I and Honek A (1996) reported that lower threshold temperature and thermal constant (k) value of a species is usually determined in the form of their emergence, growth and seasonal abundance in a given circumstances. Papanikolaou *et al.* (2014) reported that the thermal summation is useful in predicting the phenology of Coccinellid beetles. If the environmental conditions in the targeted area are similar to desirable conditions, then the chances of its establishment increase. The predatory potential of different larval instars was significantly affected by temperature. Among different larval instars the most voracious stage was fourth instar larvae. The predatory potential of fourth instar larvae was (482.4±5.37) aphids at 24 ±1 °C. The maximum total larval predatory potential was (738.9±8.92) at 24 ±1 °C. The highest predatory potential of male and female beetles was (11865±276.28 and 12986±249.2) at 24

±1 °C. Yu *et al.*, (2013) reported that the total number of aphids (*Aphis gossipy*) consumed by larvae of *H. dimidiata* was 2877.3, 3081.9 and 1722 at 15, 20 and 25 °C respectively. The total predatory potential of male beetle was 19690 and female beetle was 18355 at 25 °C. The results demonstrate that extreme low and high temperature levels have deteriorated effect on the prey consumption capacity of larval instars as well as adult male and female beetles. The daily predatory potential of different stages was also significantly different at different temperatures. Maximum potential/day was observed for female beetle at 24 ±1 °C. Similarly among different larval instars, maximum potential/day was recorded for fourth instar as compared to other larval instars. W. A Gillani *et al.*, (2007) reported that the predatory potential of female beetle was maximum and among the larval instars, fourth instar larvae consumed maximum aphids, when fed on *B. brassicae* aphid. Yu *et al.*, (2013), reported that male beetle consumed maximum aphids as compared to female beetles, when fed on *A. gossipy* aphid. The same authors also indicated that larvae consumed maximum aphids at 15 °C as compared to 25 °C and the larval potential is very high as compared to the current results. These variations may be due to different aphid species, prey stage and size or may be because of different strain and biotype of *H. dimidiata* they used in the experiments.

Table 1: Developmental durations and percent survival of *H. dimidiata* immature stages reared on *R. padi* aphid at five constant temperatures with 16:8 (L: D) photoperiods

Development Stage	Temperature														
	16±1 °C			20±1 °C			24±1 °C			28±1 °C32±1 °C			32±1 °C		
	N	%Survival	Durations (days) ± SE	N	% Survival	Durations (days) ± SE	N	% Survival	Duration (days) ± SE	N	% Survival	Duration (days) ± SE	N	% Survival	Duration (days) ± SE
Egg	100	78.0	9.06±0.07a	100	89.0	5.18±0.07b	100	94.0	4.07±0.06c	100	75	3.195±0.08d	100	60	3.02±0.08d
1 st instar	78	85.8	3.91±0.09a	89	86.6	3.0±0.08b	94	93.7	2.45±0.08c	75	85.4	2.01±0.08d	60	65	1.97±0.11d
2 nd instar	67	85.1	3.82±0.05a	77	94.9	3.0±0.08b	88	97.8	2.36±0.06c	64	89.1	2.01±0.07d	39	79.5	1.82±0.09d
3 rd instar	57	89.5	6.4±0.12a	73	91.8	5.4±0.13b	86	97.7	4.71±0.06c	57	89.5	3.51±0.09d	31	77.5	2.42±E0.07e
4 th instar	51	94.2	15.65±0.39a	67	95.6	11.2±0.12b	84	98.9	9.07±0.12c	51	92.2	6.42±0.12d	24	87.5	4.0±0.09e
Larvae	51	57.7	29.78±0.67a	67	75.3	22.61±0.38b	84	89.4	18.52±0.29c	51	68.0	14.0±0.14d	21	35.0	10.21±0.17e
Pre pupa	48	95.9	2.01±0.04a	64	92.2	1.29±0.04b	83	97.6	1.02±0.03c	47	82.9	1.01±0.04c	16	80.9	1.0±0.03c
Pupa	46	97.9	10.02±0.06a	59	94.9	6.91±0.09b	81	96.3	5.14±0.09c	39	82.1	4.0±0.09d	15	76.2	3.38±0.13e
Egg-Adult	45	45.0	50.87±0.75a	56	56.0	35.99±0.39b	78	78.0	28.75±0.47c	32	32.0	22.2±0.12d		15.0	17.62±0.23e
M/F Ratio	21/24 1:1.04			23/33 1:1.10			37/41 1:1.04			16/20 1:1.04			6/8 1:1.02		

Means within the rows with different lowercase letters are significantly different from each other at P value ≤ 0.05 (one-way ANOVA) Tukey HSD test, N= Number of initial insets used for each stage

Table 2: Biological parameters of adults male and female *H. dimidiata* fed on *R. padi* aphids at five constant temperatures with 16: 8 (L: D) photoperiods

Temp.	N Male/ Female	Pre-oviposition period±SE	oviposition period±SE	Post oviposition period±SE	Adult longevity ±SE		Total eggs per female ±SE	Eggs per female/day±SE
					Female	Male		
16±1 °C	21/24	21.8±0.39a	49.4±1.14a	15.2±0.47a	86.4±1.08a	78.35±1.28a	267.4±0.88a	5.41±0.09a
20±1 °C	23/33	13.7±0.19b	41.2±0.62b	11.0±0.42b	65.9±0.77b	61.57±1.57b	413.8±6.68b	10.04±0.07b
24±1 °C	37/41	9.4±0.17c	34.0±0.45c	9.5±0.37a	52.9±0.65c	48.67±0.72c	483.2±5.47c	14.21±0.09c
28±1 °C	16/20	7.0±0.37d	23.4±0.67d	7.5±0.38d	37.9±1.03d	33.63±0.88d	141.4±3.41d	6.04±0.15d

Means within the columns with different lowercase letters are significantly different from each other at P value ≤ 0.05 (one-way ANOVA) Tukey HSD test, N= Number of insects used

Table 3: Linear regression equations, lower developmental thresholds (T_0) and thermal constant (K) of different developmental stage of *H. dimidiata* reared on *R. padi* aphid

Dev. Stages	Regression equation	r ²	P	T ₀ (°C)	K (degree days, DD)
Egg	Y=0.056x+0.07	0.964	0.0029	-1.25 °C	17.86 °D
Larva	Y=.015x+.013	0.952	0.0045	-0.86 °C	66.6 °D
Pupa	Y=0.049X+.047	0.998	0.0000	-0.96 °C	20.41 °D
Egg to adult	Y=0.009X+0.009	0.989	0.0005	-1 °C	11.1 °D
Female beetle	Y=0.008x+0.00	0.873	0.0199	-0 °C	142.86 °D
Male beetle	Y=0.008x+0.00	0.895	0.0149	-0 °C	123.46 °D

Parameters estimated by plotting developmental rates ($y= 1/D$, development duration in days) against temperature (x), Where T_0 = Lower threshold for development, that was calculated as x- intercept (-a/b) of the linear regression model and K = Thermal constant, calculated as 1/b of the regression model

Table 4: Predatory potential of *H. dimidiata* larvae and adult male and female beetles fed on *R. padi* aphid at five constant temperatures

Temperature	1 st instar potential	2 nd Instar Potential	3 rd Instar Potential	4 th instar potential	Total grub predatory potential	Adult female predatory potential	Adult male predatory potential
16 ±1 °C	34.600A±0.7758	48.40A±0.7619	89.80A±1.3909	394.2A±5.2517	567.0A±14.045	8766.6A±85.544	8406.0C±239.99
20 ±1 °C	46.20B±0.5611	65.40B±0.8162	123.80B±1.7795	456.5B±8.5445	691.9B±9.0092	10318B±265.87	9777.1B±280.13
24 ±1 °C	52.40C±0.7886	71.6C±1.8372	142.90C±1.5843	482.4C±5.3736	738.9C±8.9127	12986C±249.26	11865A±276.28
28 ±1 °C	38.60D±0.7343	47.3A±0.8278	84.0D±1.4527	338.2D±3.8496	508.1D±6.3870	7394.8D±188.56	6813.2C±235.90
32 ±1 °C	21.0E±0.4987	38.4D±0.7227	60.2E±	212.1E±1.7930	324.84E±5.3161	2261.8E±130.08	2512.9D±131.80
LSD (0.05)	1.9025	3.0116	3.9553	15.149	25.806	551.04	670.79

Means within the columns with different lowercase letters are significantly different from each other at P value ≤ 0.05 (one-way ANOVA, LSD5%value)

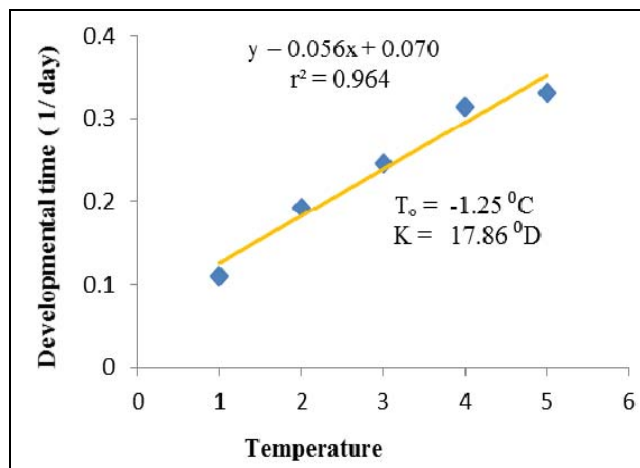


Fig 1: Effect of temperature on the development rate of egg stage

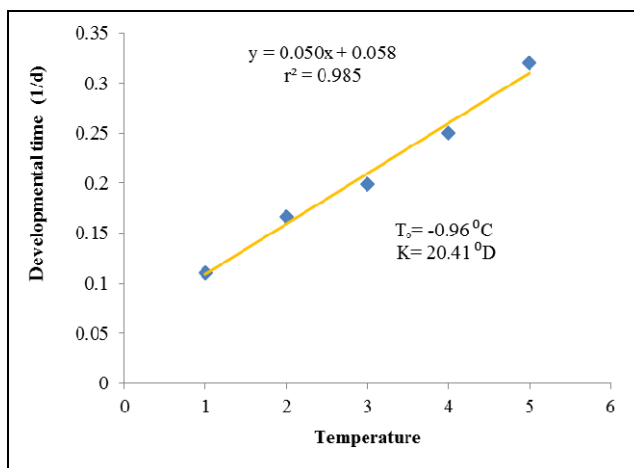


Fig 3: Effect of temperature on the development rate of pupa

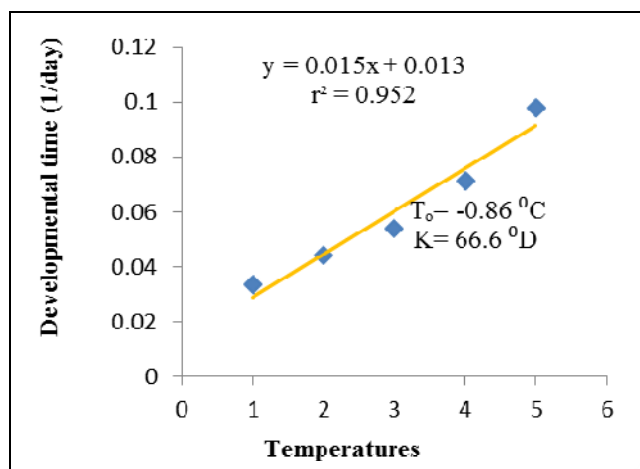


Fig 2: Effect of temperature on the development rate of larvae

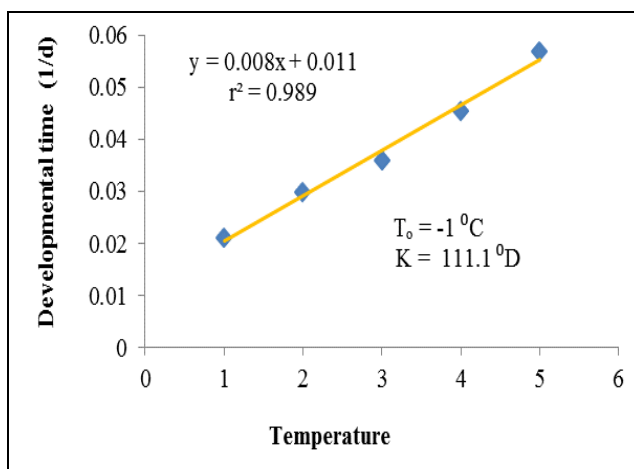


Fig 4: Effect of temperature on the development rate from egg to adult emergence

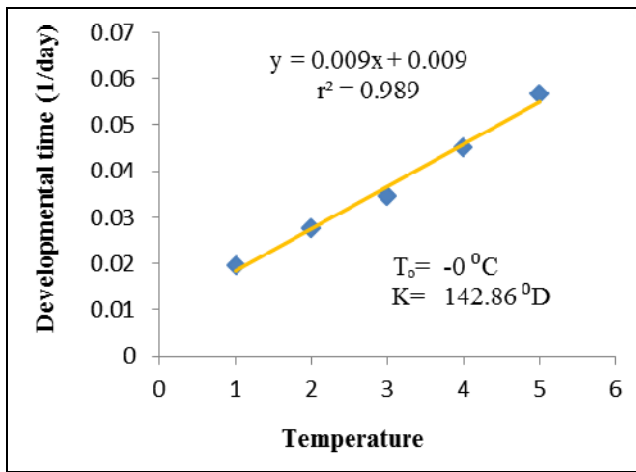


Fig 4.1: Effect of temperature on the development rate of adult female

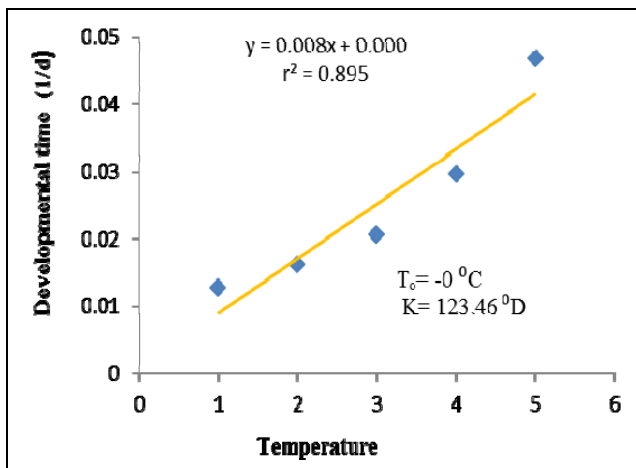


Fig 4. 2: Effect of temperature on the development of adult male beetle ladybird beetle

Conclusion

On the basis of the present studies, it was concluded that out of all tested temperature regimes (24 ± 1 °C) is the optimum temperature for the rearing of *H. dimidiata*, fed on *R. padi* aphid, owing to their shorter developmental time, high survival rate and maximum reproductive and predatory potential. The results indicate that temperature has significant effect on the biology and predatory potential and with increasing temperature the durations of different stages decreased significantly. Both extreme low and high temperatures have deteriorated effect on survival of immature stages, fecundity and predatory potential. At extreme high temperature 32 ± 1 °C, the female could not produce eggs. Considering the importance of *H. dimidiata* further studies should be undertaken, including field studies, for better understanding of its biology and ecology as well as its function in the biological control of economically important aphid pests.

Acknowledgements

The research article is PhD research work and the author highly acknowledges the cooperation and support of supervisory committee for their full time cooperation and valuable suggestions during the whole experimental duration.

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