Influence of Varying Temperature on Life Stages of *Chrysoperla carnea* (Stephens) under Laboratory Conditions

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Abstract: A laboratory study was conducted to evaluate the influence of different temperatures on life stages of *Chrysoperla carnea* (Stephens) on frozen eggs of *Sitotroga crealella*. The study was conducted under laboratory conditions at Department of Plant Protection, SAU, Tandojam Sindh, Pakistan during 2013-14. The result revealed that the maximum hatching 88 % of eggs was recorded at 28 ºC followed by 25, 31, 22, 34 and 37 ºC. The highest mortality (dx) was recorded in first instar at 34 ºC followed by at 22, 31, 37, 25 and 28 ºC, whereas, minimum (dx) was recorded in third instar and pupal stages as well. The highest and lowest apparent mortality (100qx) was observed in the first instar and egg stages at 37 ºC. The data further depicted that the highest survival fraction (Sx) was recorded as (0.98) in second instar, third instar and pupa at 28 ºC, whereas, the lowest (Sx) was observed as (0.13) in the first instar at 37 ºC. The maximum indispensable mortality (IM) was 42 in egg stage at 37 ºC and lowest 1.0 in pupal stage at all temperature regimes. The number of the surviving at the beginning of the stage (lx) was highest 39 adults emerged at 28 ºC followed by 31, 24, 20 and 5 adults emerged at 25, 31, 22 and 34 ºC, respectively. On the other hand, minimum total generation mortality (K) was recorded as 0.11 at 28 ºC followed by 0.21, 0.32, 0.40, and 1.00 at 25, 31, 22 and 34 ºC, respectively. The minimum duration from egg to adult emergence was 12.0 days at 34 ºC and maximum 23.5 days at 22 ºC. There was significant difference between the duration and treatments (*P<0.05*). It is concluded that the maximum mortality was recorded at 37 ºC in egg and first instar stages, no any stage was survived after second instar. The temperature ranges from 25 to 31 ºC have been proved suitable for the development of *C. carnea.*. However, 1st and 2nd instar survived for short period at 37 ºC only.

Keywords: *Chrysoperla carnea*, *Sitotroga cerealella*, Life stages, Development time, Temperature.

INTRODUCTION

Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) and closely related species are generalist predators found all over the world. They are sold commercially by many producers [1-3] to keep insect pest population below Economic Threshold Level (ETL). Introduction and release of *C. carnea* can contribute to effective biological insect pest control. Larvae feed on a variety of soft bodied arthropods pests i.e. whiteflies, caterpillars, thrips, leafhopper, and aphids [4, 5]. Lacewing larvae have been recorded to eat 100 to 600 aphids each [6, 7]. Because of the high food searching ability, relatively fast reproduction and the ability to keep several pest species below the ETL, *C. carnea* is a very useful insect as biological control agent on advanced IPM techniques in agricultural fields [8]. Populations of sucking and chewing pests can be reduced by applying cards with egg of green lacewings, which can be used in vegetables, cotton, and different fruit crops [9-12]. At low (13 ºC) and high (33 ºC) temperatures, however, flying activity of *C. sinica* has been shown to be low. Also relative humidity (30% RH) reduced lacewing performance [13]. Mass Rearing of *C. carnea* can easily be done in the laboratory [14]. Fecundity, developmental period and survival rate of *C. carnea* are depending on biotic and abiotic factors [15]. The most important environmental factor, however, is temperature as it influences the developmental rate of a particular insect species [16]. The experiment presented here was conducted during 2014 in the laboratory of the Plant Protection Department, Sindh Agriculture University, Tandojam, Pakistan. The aim was to investigate the influence of varying temperature on life stages of *C. carnea* (Stephen) under laboratory conditions. This information is important for entomologists, ecologists, and particularly for farmers and mass production laboratories to optimize the effective release of lacewings for pest control.

MATERIALS AND METHODS

The influence of different temperatures on the developmental stages of *C. carnea* was determined. Survival and developmental times of *C. carnea* at different temperatures was evaluated. The eggs of *C. carnea* were collected from the Nuclear Institute of Agriculture (NIA) Tandojam. For the experiment, 50 eggs were placed in separately in glass vials (10×1 cm). The neonates were fed with frozen eggs of

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Sitotroga cerealella (Lepidoptera: Gelechiidae) The different temperature treatments were: T_1=22 ºC, T_2=25 ºC, T_3=28 ºC, T_4=31 ºC, T_5=34 ºC, and T_6=37 ºC. Experiments were conducted in refrigerated incubator (Model No. FOC 2251 made by VELP Scientifica, Europe). On a daily basis, mortality and development into the different instars, Pupae, and adults were monitored. A photo period 10 hours light and 14 hours dark and the relative humidity was maintained. The experiment was stopped after adult emergence when the sex ratio was recorded. The collected data were subjected to analyses according to this formula:

Stage Specific Life-Table

The stage specific survival and mortality of eggs, larvae, pupae and adults of *C. carnea* were recorded:

x = stage of insect,

1x = number of individuals at the beginning of the stage x,

dx = number of dying individuals during the stage x.

The following parameters were calculated:

Apparent Mortality (in percent) = [dx / lx] ×100

Survival Fraction of particular stage (Sx) = [lx of subsequent stage] / [lx of particular stage].

Mortality Survivor Ratio (MSR) of particular stage = [staged of particular]/ [lx of subsequent stage]

Indispensable Mortality (IM) = [Number of adults emerged] x [MSR. of particular stage]

K-Values

The total generation mortality was calculated by adding the k values of the different development stages of the insect, which is indicated as "K" [17, 18]:

$$K = k_E + k_{L1} + k_{L2} + k_{L3} + k_P$$

The different k values represent eggs, first instar, second instar, third instar, and pupae of *C. carnea.*

Total Life Span of Immature Stage

The total life span of *C. carnea* was calculated by adding the life period of each life stage i.e. life span of egg, first instar, second instar, third instar larvae and pupa.

RESULTS

The result achieved in this study are given in Table **1** described below.

Apparent Mortality

In general, the maximum apparent mortality was at egg stage with 84.0 % at 37 ºC, followed by 52, 30, 24, 20 and 12 % mortality at 34, 22, 31, 25 and 28 ºC, respectively. At 37°C, all hatching larvae (except one) died in the first instar. In the other temperature treatments, the lowest mortality in the first instar (4.55 %) was recorded at 28 ºC followed by 12.50, 18.42, 28.57, 50.0 at 25, 31, 22, and 34 ºC, respectively. In the 2nd instar, the highest mortality (41.67 %) was observed at 34°C, larvae followed by 12, 9.68, 5.71 and 2.38 % at 22, 31, 25 and 28 ºC, respectively. Similarly, the highest mortality at 3rd instar (14.29 %) was observed at 34 ºC followed by 7.14, 4.55, 3.03 and 2.44 % at 31, 22, 25 and 28 ºC, respectively. The maximum pupal mortality (16.67 %) was recorded at 34 ºC followed by 4.76, 4.0, 3.13 and 2.50 % at 22, 31, 25 and 28 ºC, respectively. The predator was not able to complete its life cycle at 37 ^{0}C, as all individuals (except one) died as eggs or first instars.

Mortality Survivor Ratio (MSR)

The result depicted in Table **1** that the maximum mortality survivor ratio was obtained at egg stage 5.25 at 37 ºC followed by 1.08, 0.43, 0.32, 0.25 and 0.14 at 34, 22, 31, 25 and 28 ºC, respectively. The data further revealed that highest MSR was recorded in the 1st instar larvae 7.0 at 37 ºC followed by 1.0, 0.40, 0.23, 0.14 and 0.05 at 34, 22, 31, 25 and 28 ºC, respectively. Similarly, in the 2nd instar larvae showed highest MSR was 0.71 at 34 ºC followed by 0.14, 0.11, 0.06 and 0.02 at 22, 31, 25 and 28 ºC, respectively. The highest MSR was observed in the 3rd instar larvae 0.17 at 34 ºC followed by 0.08, 0.05 and 0.03 at 31, 22 and 25 as well as 28 ºC, respectively. It is noticed from the above described results that the highest MSR was seen in the 3rd instar larvae at 37 ºC and lowest was 0.03 at 25 as well as 28 ºC. The data further indicated that the maximum MSR was determined in the pupal stage 0.20 at 34 ºC followed by 0.05, 0.04 and 0.03 at 22, 31 and 25 as well as 28 ºC, respectively.

Indispensable Mortality (IM)

The result in Table **1** depicted the highest indispensable mortality IM was seen 42 at egg stage, at the 37 ºC followed by 26, 15, 12, 10 and 6 was

Table 1: Effect of Varying Temperature on the Life Stages of *Chrysoperla carnea* (Stephens) Fed on *Sitotroga crealella* in the Laboratory

Stage x	No. surviving at the beginning of stage lx	No. dying in each stage dx	Apparent mortality 100qx	Survival fraction Sx	Mortality survivor ratio MSR	Indispensable mortality IM	Log lx	k-Value
Temp: 22^{0}C								
Egg	50	15	30.00	0.70	0.43	15.00	1.70	0.15
First instar	35	10	28.57	0.71	0.40	10.00	1.54	0.15
Second instar	25	3	12.00	0.88	0.14	3.00	1.40	0.06
Third instar	22	1	4.55	0.95	0.05	1.00	1.34	0.02
Pupa	21	1	4.76	0.95	0.05	1.00	1.32	0.02
Adult	20						1.30	
								K=0.40
Temp: 25^{0}C								
Egg	50	10	20.00	0.80	0.25	10.00	1.70	0.10
First instar	40	5	12.50	0.88	0.14	5.00	1.60	0.06
Second instar	35	2	5.71	0.94	0.06	2.00	1.54	0.03
Third instar	33	1	3.03	0.97	0.03	1.00	1.52	0.01
Pupa	32	1	3.13	0.97	0.03	1.00	1.51	0.01
Adult	31						1.49	
								K=0.21
Temp: 28^{0}C								
Egg	50	6	12.00	0.88	0.14	6.00	1.70	0.06
First instar	44	2	4.55	0.95	0.05	2.00	1.64	0.02
Second instar	42	1	2.38	0.98	0.02	1.00	1.62	0.01
Third instar	41	1	2.44	0.98	0.03	1.00	1.61	0.01
Pupa	40	1	2.50	0.98	0.03	1.00	1.60	0.01
Adult	39						1.59	
								K=0.11
Temp: 31^{0}C								
Egg	50	12	24.00	0.76	0.32	12.00	1.70	0.12
First instar	38	7	18.42	0.82	0.23	7.00	1.58	0.09
Second instar	31	3	9.68	0.90	0.11	3.00	1.49	0.04
Third instar	28	2	7.14	0.89	0.08	2.00	1.45	0.05
Pupa	25	1	4.00	0.96	0.04	1.00	1.40	0.02
Adult	24						1.38	
								K=0.32
Temp: 34^{0}C								
Egg	50	26	52.00	0.48	1.08	26.00	1.70	0.32
First instar	24	12	50.00	0.50	1.00	12.00	1.38	0.30
Second instar	12	5	41.67	0.58	0.71	5.00	1.08	0.23
Third instar	7	1	14.29	0.86	0.17	1.00	0.85	0.07
Pupa	6	1	16.67	0.83	0.20	1.00	0.78	0.08
Adult	5						0.70	
								K=1.00

(Table 1). Continued.

Stage x	No. surviving at the beginning of stage lx	No. dying in each stage dx	Apparent mortality 100qx	Survival fraction Sx	Mortality survivor ratio MSR	Indispensable mortality IM	Log lx	k-Value
Temp: 37^{0}C								
Egg	50	42	84.00	0.16	5.25	42.00	1.70	0.80
First instar	8	7	87.50	0.13	7.00	7.00	0.90	0.90
Second instar	1	0	0.00	0.00	0.00	0.00	0.00	0.00
Third instar								
Pupa								
Adult								
								K=1.70

observed at 34, 22, 31, 25 and 28 ºC, respectively. The data further revealed that the minimum IM was recorded 2 at 28 ºC followed by 5, 7, 10 and 12 at 1st instar larval stage was recorded at 25, 31, 22, and 34 ºC, respectively. Therefore, in the 2nd larval instar showed maximum IM was recorded 5 at 34 ºC followed by 3, 2 and 1 at 22 ºC as well as 31 ºC, 28 and 25 ºC, respectively. The 3rd larval stage showed the highest indispensable mortality 2 at 31 ºC and lowest was 1 at 22 ºC as well as 25, 28, and ºC. The highest indispensable mortality was observed in 1st instar larvae at 34 ºC and lowest in the 3rd instar larvae at 22, 25, 28 and 34 ºC. The result further depicted that the indispensable mortality was recorded 1 at 22, 25, 28, 34 and 37 ºC in the pupal stage.

k-Value

The data given in Table **1** showed that the minimum k value at egg stage was observed 0.06 at 28 ºC followed by 0.10, 0.12, 0.15, 0.32 and 0.83 at 25, 31, 22, 34, and 37 ºC, respectively. The result further indicated that the maximum k value was recoded at 1st instar larval stage 0.90 at 37 ºC followed by 0.30, 0.15, 0.09, 0.06 and 0.02 at 34, 22, 31, 25 and 28 ºC, respectively. However, in the 2nd instar larvae the highest k value was observed 0.23 at 34 ºC followed by 0.06, 0.0.04, 0.03 and 0.01 at 22, 31, 25 and 28 ºC, respectively. Similarly, the highest k value was recorded in the 3rd instar larvae 0.07 at 34 ºC followed by 0.05, 0.02 and 0.01 at 31, 22 and 25 as well as 28 ºC, respectively. It was observed that the maximum k value was seen in the 1st instar larvae at 37 ºC and lowest was in 3rd instar stage at 25 as well as 28 ºC. The result further revealed that the maximum k value was seen in the pupal stage 0.08 at 34 ºC followed by 0.02, and 0.01 at 22 as well as 31 and 25 as well as 28 ºC, respectively. The lowest K value was recorded 0.11 at 28 ºC followed by 0.21, 0.32, 0.40, 1.0 and 1.70 at 25, 31, 22, 34 and 37 ºC, respectively.

Development of Immature Stages of *C. carnea*

The results depicted in Table **2** show that the minimum egg incubation period observed was 1.0 days at 37 ºC followed by 2.0, 2.5, 3.0, 4.0 and 4.5 days at 34, 31, 28, 25 and 22 ºC, respectively. The minimum development period in the 1st instar larvae was 1.0 days recorded at 37 ºC followed by 1.5, 2.0, 3.0 and 3.5 days at 34, 31, 28 and 25 as well as 22 ºC, respectively. Similarly, 2nd instar larvae showed a minimum developmental period of 1.0 days at 37 ºC followed by 2.0, 2.5, 3.0 and 3.5 days at 34, 31, 28 and 25 as well as 22 ºC, respectively. The minimum developmental period in the 3rd instar larvae recorded was 2.0 at 34 ºC followed by 3.0, 3.5, 3.0 and 4.0 days at 31, 28, 25 and 22 ºC, respectively. The result further revealed that total minimum larval development was observed 5.5 days at 34 ºC followed by 7.5, 9.5, 10.0 and 11.0 days at 31, 28, 25 and 22 ºC, respectively. The minimum pupal development period was recorded 4.5 days at 34 ºC followed by 5.5, 8.5, 9.5 and 8.0 days at 31, 28, 25 and 22 ºC, respectively. The result further revealed that the total minimum duration from egg to adult emergence was seen 12.0 days at 34 ºC followed by 15.5, 21.0, 23.5 and 23.5 days at 31, 28, 25 and 22 ºC, respectively. It was observed that at 37 ºC only 1st and 2nd instar survived for short period although it was also observed that highest temperature ranges showed shortest development period. Analysis of variance showed that there were highly significant difference between days and treatments (P<0.05).

Table 2: Mean Development Time in Days of Immature Stages of *Chrysoperla carnea* at Varying Temperatures

Temperature	Development time of immature stages(days)						
	Incubation period	1st Instar	2nd Instar	3rd Instar	Total Larval period	Pupal period	Duration from egg to adult emergence
22 ºC	4.5 a	3.5 a	3.5 a	4 0 a	11.0	8.0 b	23.5 a
25 ºC	4.0 ab	3.5 a	3.5 a	3.0 c	10.0	9.5 a	23.5 a
28 ºC	3.0 c	3.0 b	3.0 b	3.5 b	9.50	8.5 ab	21.0 b
31 ºC	2.5 cd	2.0 c	2.5 c	3.0 c	7.50	5.5 c	15.5 c
34 ºC	2.0 d	1.5 d	2.0 d	2.0 d	5.50	4.5 cd	12.0 d
37 ºC	1.0 e	1.0 e	1.0 e	0.0 e	0.00	0.0 e	0.0 e

Different letters within a row indicate significant difference (Fisher's Protected LSD test: *P< 0.05*).

DISCUSSION

The results of the present study revealed that the maximum survival from egg to adults (78%) was at 28 ºC, followed by 62% at 25°C. In the 22°C and the 31°C treatments, 40% and 48% of the eggs became adults. No development to adulthood was possible at 37 ºC and only 10% of the eggs developed into adults at 34°C. The egg incubation period, developmental period of immature stages and the pupal period in days was decreasing with increasing temperature. Our results are similar with those reported by Khan *et al*. [19] who reported developmental periods of immature stages of *Chrysoperla carnea*, feeding on *Corcyra cephalonica* (Lepidoptera: Pyralidae) eggs at three constant temperatures, i.e. 24 ±1 ºC, 28 ±1 ºC and 32 ±1 °C. The incubation period of egg was 4.9±0.08, 3.8±0.08 and 3.0±0.06 days, respectively. Thedurations of the first instar were 3.6±0.07, 3.0±0.11, and 2.0±0.06 days, of the second instar 3.4±0.11, 3.0±0.07, and 2.8±0.07, and the third instar 4.9±0.10, 4.0±0.06, and 3.4±0.13 days at the three temperatures ranges, respectively. Our findings partially align also with Sharma *et al.* [20], who reported the development of *C. carnea* at 15, 25 and 30°C. The mean incubation period at 30°C was recorded as 2.8 days. At 25°C, the incubation period was 3.7 days. The average egg hatching at 30°C was 48%. At 25°C, an average of 64% egg hatched. A total of 56% of the eggs hatched at 15°C. The average larval period of the first instar at 30°C was 2.9 days. The results of our study are partially supported by the results of Bezerra *et al.* [21] who investigated that survivor, mortality and development of life stages of green lacewing, *Chrysoperla genanigra* varied significantly at constant temperatures (17, 21, 25, 29, 33, 35 and 37 °C), a photoperiod of 12 h: 12 h (L: D) and 70 ± 10% relative humidity.

In conclusion, our study demonstrates that survival of *C. carnea* in the laboratory was optimal at 28°C. Higher temperatures increased mortality with very low or no survival at 34 and 37°C, respectively. Lower temperatures also increased mortality to some extend and development was somewhat slower.

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