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Effect of temperatures on development, survival, sex ratio, storage of eggs and pupae of *Apertochrysa* sp. and post-storage effects on pupae

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Key words: Green lacewing, *Apertochrysa*, Disability, Survival, Sex ratio, Storage.

Abstract

The effect of temperature were evaluated under 10°C,15°C, 20°C, 25°C and 28°C to determine the optimum rearing condition of green lacewing *Apertochrysa* sp. The highest developmental periods of eggs (10.45 days), larval first instars (13.63 days), larval second instars (17.18 days), larval third instars (19.72 days) and pupal stages (27.36 days) occurred under 15°C while the lowest developmental periods of eggs, larval first instars, larval second instars and larval third instars are 4, 4, 3.6, and 3.7 days respectively were obtained at 25°C. No any development of *Apertochrysa* sp. at 10°C was noticed. The highest survival (55%) was recorded at 15°C. The sex ratio of emerging adults from four temperatures did not differ significantly with temperatures from 1:1 except in case of laboratory condition. Zero percentage of eggs hatchability was noticed when the eggs were stored at 10°C. The maximum storage period was 9.49 days at 15°C with high survival of eggs 76.51 %. The highest percentage of survival of pupae stored of *Apertochrysa* sp. was recorded after 10 days storage at 10°C (94.1 %) but it's not differ significantly with survival of pupae stored 20 days at 20°C (83.77 %). The lowest disability of adults was recorded by nonstored pupae at 25°C (7.6 %). The longest pre-emergence period 13.20 days was recorded under 20 days pupal storage at 10°C. The high percentages of good quality adults were obtained from pupae stored short storage period (10 days) at 10°C (85.39%) and 15°C (81.81%).

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Introduction

The green lacewings (Neuroptera: Chrysopidae) are an important group of insect predators (Dean and Satasook, 1983). It has agronomical importance (Ohm and Holzel, 2002). The recently recorded green lacewing *Apertochrysa* sp. from agro-ecosystem in Malalysia can play a very important role in Malaysian agriculture (Alasady *et al*., 2010). The production of large numbers of natural enemies of high quality and the cost–effective rearing techniques are needed for augmentative biological control (Riddick, 2008), and the efficient mass production of the control agent is crucial to the success of any control program (Tulisalo, 1984**).** Continuous and large supply of insects is a pre-requisite for entomological research and the large number of insects can be reared under controlled conditions (Dhandapani *et al*., 1992).

Environmental factors like temperature, photoperiod and humidity are important factors for determination of their rearing conditions and can affect the development, survival and diapauses of insects. (Nakahira *et al*., 2005).

Temperatures can affect developmental, survival of immature, reproductive and adult longevity of green lacewing (Silva *et al*., 2007; Nakahira *et al*., 2005; Pappas *et al*., 2008). However, there is little information available regarding thermal effects on development and survival of green lacewing (Liu, 1989; Silva, 2007).

Storage was identified by King and Jackson (1985) as; the storage of entomophagous without loss of viability and effectiveness. The ability to store entomophagous is the key factor for successful biological control programs (Morrison and King, 1977). Efficient storage with good post storage effects on larvae and adults is crucial for commercialization and effective use of natural enemies in development of augmentation methods of biological control (Lopez-Arroyo *et al*., 2000; Tezze and Botto, 2004), making the biological control more economically attractive (Dent and Walton, 1997). Good storage techniques can provide flexibility in mass production and offer the availability of natural enemies in sufficient number at the time of release (Tezze and Botto, 2004; Greenberg *et al*., 1996).

The potential storage techniques for green lacewings have been done by utilization the diapause stage and low temperatures (Osman and Selman, 1993; Tauber *et al*., 1997) and the storage of various chrysopids predators was made often with eggs and pupae (Nordulund and Morrison, 1992). While the eggs and young larvae at most were used for green lacewings releases (Osman and Selman, 1993).

However there are many factors that can affect the storage term of natural enemies like the specific stage, age of this stage (Ayvaza *et al*., 2008) or may be the type of the food. The development, survival and diapause of insects are highly affected by temperature and photoperiod, thus the study of thermal effects is important for rearing and storage of biological control agents (Nakahira *et al*., 2005; Silva *et al*., 2007). Although the storage effect of many chrysopids species was known like *Ceraochrysa cubana* (Hagen), *Ce. smithi* (Navas), *Chrysoperla externa* (Hagen) (Lopez-Arroyo *et al*., 2000) and *Dichochrysa parsina* (Burmeister) (Pappas *et al*., 2008), still the storage effects on the various stages of many green lacewings species are unknown and the *Apertochrysa* sp. is one of them. The *Apertochrysa* sp. is a promising predator and newly recorded in Malaysia (Alasady *et al*., 2010) which needs extra studies for using it as biological control agent. The storage techniques of *Apertochrysa* sp. were needed for future biological control programs.

The aim of present work is to study the effects of temperatures on preimaginal development, survival and sex ratio of the green lacewing *Apertochrysa* sp., also to optimize the suitable temperature for rearing and to determine the temperatures to be used for storage and to determine possibility of storing the eggs and pupae of *Apertochrysa* sp., study the effect of period's storage and post storage effects of eggs and pupae at various temperatures. Hatchability of eggs stored, maximum storage period, Mortality of

pupae stored, survival of pupae stored, disability of adults emerged from pupae stored, pre-emergence time and final good quality adults produced from pupae stored were studied.

Materials and methods

Rearing of C. cephalonica

Sterilized maize, rice, wheat and semolina (1:1:1:1) were placed in a plastic cage. Eggs of *Corcyra cephalonica* were spread over the diet inside the cage at 22ºC, 55-85% RH, and 12L: 12D photoperiod to develop *C. cephalonica* to adult stage that was collected for matting in plastic cage. The eggs produced were collected in a glass plate. This plate was placed in the freezer to exhaust egg viability. These eggs were used as food for larvae of *Apertochrysa* sp.

Rearing of Apertochrysa sp

The laboratory culture of *Apertochrysa* sp. was established from eggs which were collected from citrus orchards in UPM fields, Serdang /Malaysia, and were reared at (25 ± 1ºC, 55-85% RH, and 12L: 12D photoperiod) conditions. The newly emerged larvae were fed on frozen *C. cephalonica* eggs individually to prevent cannibalism in plastic Petri dishes (10cm dia. x 1cm height) until pupate. The adults that emerged from pupae in the Petri dish were sent to oviposition plastic cage (37x28cm dia. and 22cm height) covered with black organza cloth, and provided daily with an artificial diet (3gm sugar, 2.5gm yeast, 2.5ml honey, 3gm powder milk (instead of casein) by smearing the wall of the cage with the artificial diet daily using special brush.

Effect of temperature on preimaginal developments, survival and sex ratio of Apertochrysa sp.

Thermal effects on developments, survival and sex ratio were examined at five constant temperatures 10ºC, 15ºC, 20ºC, 25ºC and laboratory condition (28ºC±3ºC) with 55-85% RH, and 12L: 12D photoperiod regime. The first laboratory generation eggs within 24h were used in the experiments. The 60, 20, 36, 116, and 148 eggs were individually transferred to plastic Petri dishes (10cm dia. x 1cm height) with a lid to prevent the larvae cannibalism, were provided with enough quantity of frozen *C. cephalonica* eggs and maintained under the five different temperature regimes respectively. Daily observations were made on survival and development until emergence of adults.

The number of eggs hatchability, development period, mortality rate in each immature stage and adult's sex ratio were recorded. Eggs that had not hatched and pupae. within the cocoon that had not emerged within 2 month from the start of the experiment were regarded as dead.

Storage of eggs and pupae

The first laboratory generation eggs of *Apertochrysa* sp. laid and pupae formed within 24h were used in the experiments. Three groups of eggs were placed in plastic Petri dishes (10cm x 1cm dia.) and three groups of pupae (each pupa in each Petri dish) as three replicates in completely randomize design (CRD) as orthogonal comparison of each storage period of 10, 20, 30, 40, 50, and 60 days were stored at three different temperatures regimes of 10ºC, 15ºC, 20ºC and 25ºC as a control. Each eggs and pupae groups of each storage period were transferred to 25ºC after storage. The hatches of eggs, the maximum eggs hatch times, adult's emergences, pupae mortality and adults' disability of all treatment were monitored and recorded daily. Eggs that had not hatched and pupae that had not emerged within three months from last day of storage were regarded as dead.

All percentages were calculated as follows:

Number of hatch eggs

a. The percentages of eggs hatch = --- x 100 Total number of eggs exposed to storage

Number of nonemerged pupae

b. The percentages of pupae mortality =--- x 100
Total number of pupae exposed to storage

c. The percentages of survivals of pupae = 100 - the percentage of pupae mortality. The percentages of disability = The number of disables individuals of adults --- x 100. Total number of adults emerged from pupae exposed to storage

d. The percentages of good quality adults = percentage of survival – the number of disables adults). Percentage of disability The numbers of disables adults (----x100) were got for calculate Survival the percentage of good quality adults.

Data analysis

The effects of temperatures on the development for whole life cycle (from eggs to adult's emergence) with and within the various stages separately were analyzed by regression the mean of development and getting the equation of regression and correlation coefficient (R^2). To compare the effect of temperature on developmental time of *Apertochrysa* sp., the data was analyzed by Analysis of Variance (ANOVA) followed by Duncan test. Deviations from a 1: 1 sex ratio and the survivals were assessed with chi-square test. The effects of storage on the eggs at different temperatures and percentage egg hatch were analyzed using ANOVA procedure and then the means of hatchability was compared by Tukey test (SAS Institute 2002). The time of eggs hatch at different temperatures was tested by analyzed the means of time of eggs hatch by one-way analysis of variance (ANOVA) followed by LSD multiple range test (P = 0.05**)** for comparing between the means. The effects of storage on the pupae, the percentage of pupae mortality, adults survivals emerged from pupae stored, disability of adults and the final good quality adults produced at various temperatures and different storage periods were analyzed using ANOVA procedure. Tukey Studentized Rang (HSD) tests (P = 0.05) were used to separate the means after ANOVA procedure (SAS Institute, 2002). All percents data were subjected to arcsine transformation to satisfy assumptions of normality (Little and Hills, 1972). The pre-emergence periods were analyzed using GLM procedure and then Duncan's multiple Rang tests were used to separate the means after one-way ANOVA (PROC GLM).

Results and discussions

Effect of temperature on perimaginal developmental time, survival and sex ratio

The (table 1) indicate that the mean developmental period of eggs, three larval instars and cocoon pupae stages of *Apertochrysa* sp. were significantly affected by temperatures 10°C, 15°C, 20°C, 25°C, and laboratory condition (28°C ± 3°C). The mean developmental time of all stages and larvae instars decreased linearly and differs significantly with increasing the temperatures 10°C, 15°C, 20°C and 25°C respectively, whereas the mean developmental times increased again under laboratory condition except in case of cocoon pupae.

The total preimaginal developmental periods (from eggs laid to adult's emergence) were changed linearly against temperatures. The highest developmental periods of eggs (10.45 days), larval first instars (13.63 days), larval second instars (17.18 days), larval third instars (19.72 days) and pupal stages (27.36 days) were occurred under 15°C, while the lowest developmental periods of eggs, larval first instars, larval second instars and larval third instars are 4, 4, 3.6, and 3.7 days respectively were obtained at 25°C. The lowest developmental time of pupae were noticed at laboratory condition.

The highest total larval developmental period was recorded at 15°C (50.54 days) followed by 41.9 days at 20°C, laboratory condition (15.7 days) and lowest total period was recorded at 25°C (11.3 days). Generally the total developmental periods (eggs to adults) are 88.36 days, 68.8 days, 33.3 days and 29.5 days at 15°C, 20°C, laboratory condition and 25°C respectively. While no any development of *Apertochrysa* sp. at 10°C was noticed.

The results also show that the survival of *Apertochrysa* sp. under the three temperatures 15°C, 20°C, 25°C differed significantly from the survival at laboratory condition (d.f = 5, $\chi^2_{calc.}$= 117.805, P = 0.05). The highest survival (55%) was recorded at 15°C followed by the survival at 25°C (52.3%) and the survival 44.4% at 20°C, while the lowest percentage of survival 42.6% was recorded under laboratory condition. The sex ratio of emerging adults from three temperatures did not differ significantly with temperatures from 1:1 (d.f = 4, $\chi^2_{calc.}$= 9.484, P = 0.05) while the sex ratio differed significantly at laboratory condition (d.f = 1, $\chi^2_{calc.}$= 5.182, P = 0.05).

Table 1. Effect of constant temperatures on development and survival of *Apertochrysa* sp. with (55-85%) RH and 12L: 12D photoperiod regime.

Stages	Developmental time (days) ± SD days (number that completed the stage)					R^2 and regression equations from 10°C to 25°C
	10°C ± 1	15°C ± 1	20°C ± 2	25°C ± 1	28°C ± 3	
Egg	0.0 (60) e	10.45±1.57 (20) a	6.3 ± 0.8 (36) b	4 ± 0.5 (116) d	5.8 ± 0.5 (148) c	y = 3.225x+0.13.36 R^2 = 0.973
1st instar	0.0 e	13.63 ± 0.67 (16) a	10.3 ± 1.9 (22) b	4 ± 0.7 (88) d	6.7 ± 1.3 (86) c	y = -0.963x + 28.57 R^2= 0.969
2nd instar	0.0 e	17.18 ± 0.87 (14) a	14.4 ± 1.7 (22) b	3.6 ± 0.5 (84) d	4.4 ± 2.3 (82) c	y = -1.358x + 38.88 R^2 = 0.895
3rd instar	0.0 e	19.72 ± 0.9 (12) a	17.2 ± 2.2 (21) b	3.7 ± 0.7 (71) d	4.6 ± 0.9 (75) c	y = -1.602x + 45.58 R^2 = 0.864
Total larval dev.	0.0 e	50.54±1.57 (12) a	41.9 ± 3 (20) b	11.3 ± 1.2 (71) d	15.7 ± 2.4 (75) c	y = -3.924x + 113 R^2 = 0.905
Pupa	0.0 e	27.36±1.8 (12) a	20.6 ± 7.6 (20) b	14.2 ± 1.5 (71) c	11.8 ± 0.4 (75) d	y = 1.316x + 47.04 R^2 = 0.999
Total development	0.0 e	88.36±1.74 (11) a	68.8 ± 7.3 (16) b	29.5 ± 2.1 (61) d	33.3 ± 2.4 (63) c	y = -5.886x + 179.9 R^2 = 0.963
Survival (%)[1]	0.0 C	55 A	44.4 A	52.3 A	42.6 B	
Sex ratio F:M[2]	0.0	1.1: 0.9 N	1.1: 0.9 N	1.1: 0.9N	1.2: 0.8 S	

Within the same rows, data followed by the same small letter are not significantly different by ANOVA followed by Duncan est (P = 0.05, d.f = 4, 166, F = 626.69, 411.97, 376.84, 1353.87, 2156.86, 266.30, 2172.51, respectively), the data followed by apital different letters are significantly different by chi-square (d.f =5, $\chi^2_{calc.}$= 117.805, P = 0.05)[1], and N not differ significantly from 1:1 (d.f = 4, $\chi^2_{calc.}$= 5.972, P = 0.05)[2], S differs significantly from 1:1 (d.f = 1, $\chi^2_{calc.}$= 5.182, P = 0.05.

The results found strong influence of temperature on preimaginal developmental periods and survival of all stages of *Apertochrysa* sp. A good survival (52.3%) with shortest total developmental period (29.5 days) was recorded at 25°C. While the highest survival with shortest developmental time at 25°C of *Chrysopodes (Chrysopodes) lineafrons* and *Dichochrysa prasina* on *Anagasta kuehniella* eggs were 83% with 31.1 days and 84% with 37 days (Silva *et al.*, 2007; Pappas *et al.*, 2008) respectively. However the survival and developmental time related to various factors like the specific species (Principi and Canard, 1984), humidity and photoperiod (Pappas *et al.*, 2007). The highest survival was recorded at 15°C (55%) but with longest developmental period (88.36 days) which not differs significantly by survival at 25°C but differed significantly by developmental time. Similar results were obtaind by Nakahira *et al.*, (2005) when they studied the green lacewings *Mallada desjardinsi* and *Ch. nipponensis* at 15°C fed on frosted eggs of *Ephestia kuehniella*. Contrast results were documented by Silva *et al.*, (2007) at 14.5°C when they found zero percentage of survival and the green lacewing *Chr. lineafrons* cannot complete its life cycle at this temperature.

At laboratory condition (28°C ± 3°C) the developmental time increased again while the previous study showed that the developmental time of green lacewings continue decreasing at 28°C (Nakahira *et al.*, 2005; Pappas *et al.*, 2007; Silva *et al.*, 2007). This research results of sex ratio of emerging adults from 15°C, 20°C, 25°C did not differ significantly from 1:1, similar results were reported by Siliva *et al.* (2007)**.** However, the significant deviation of sex ratio at 28°C from 1:1 was not documented by previous studies. The developmental periods with and within stages were linearly related to temperatures which is similar to findings reported by (Pappas *et al.*, 2008; Siliva, *et al.*, 2007; Nakahira *et al.*, 2005; Lopez-Arroyo *et al.*, 2000).

Storage of eggs

The hatch results for *Apertochrysa* sp. stored eggs and maximum storage periods at 10°C, 15°C and 20°C compared with non stored eggs at 25°C are shown in (Table 2). The results indicate that the mean percentage of eggs hatchability of *Apertochrysa* sp. at 15°C (76.51) and 20°C (69.61) differed significantly with control temperature 25°C (50.47). The highest percentage of eggs hatched occurred at 15°C showed

no significant differences with eggs hatch percentage at 20°C. Zero percentage of eggs hatchability was noticed when the eggs were stored at 10°C. While high significant differences were recorded between the storage periods and the maximum storage period was 9.49 days at 15°C followed by 7.31 days under 20°C compared with 4.28 days at 25°C. The maximum developmental period of *Apertochrysa* sp. was 9.49 days at 15°C and 7.31 days at 20°C with high survival of eggs 76.51% and 69.61% which can give a limited opportunities for storing the eggs for short time. These developmental periods of eggs close to the eggs dvelopmental period of *D. prasina* and *M. desjardinsi* at 15°C (12.3 days) and 7 days at 20°C (Nakahira *et al.*, 2005; Pappas *et al.*, 2008). While Lopez-Arroyo *et al.*, (2000) delayed the developmental period of eggs of *Ce. cubana* to 14 days at 15.6°C and the developmental period of eggs of *Chr. (Chrysopodes) lineafrons* was delayed to 15.6 days at 14.5°C with a good survival rate (Silva *et al.*, 2007).

Table 2. The mean ± SD of percentage of eggs hatch of *Apertochrysa* sp. and means of hatch period at various constant temperatures**.**

Temperature	Mean percentage of eggs hatch ± SD 1	Mean of maximum hatch period ± SD 2
10°C	0.0 ± 0.0 c (60)	0.0 ± 0.0 d (60)
15°C	76.51 ± 8.17 a (135)	9.49 ± 0.77 a (101)
20°C	69.61 ± 5.17 a (120)	7.31 ± 0.8 b (84)
25°C	50.47 ± 2.27 b (66)	4.28 ± 0.08 c (33)

Within the same column, the data followed by the same letter are not significantly different on $P = 0.05$ by Tukey's Studentized Rang (HSD) (d.f = 6, P = 4, F = 57.09, M.S.D = 0.179)[1], the data followed by the same letter are not significantly different on $P = 0.05$ by L.S.D (d.f = 3, 8, F = 163.77, L.S.D = 1.05)[2] MSD = Minimum significant differences.

At 10°C all eggs stored (10-60 days) were killed and no opportunities for storage of the eggs of *Apertochrysa* sp. under this temperature. Similar results were documented by Lopez-Arroyo *et al.*, (2000) when *Ce. cubana* and *Ce. smithi* stored at 10°C, while the eggs of *Ch. externa* can be stored for 20 days at this temperature. It seems the storage capability highly varied from species to species.

Storage of pupae

The results of tests of mortality, the percentage of successful emerged adults, the percentage of disability and pre-emergence periods of *Apertochrysa* sp. which were conducted under various storage periods at 10°C, 15°C and 20°C comparing with nonstored temperature 25°C are presented in (Table 3).

The mortality and survival of pupae stored

The results of mortality of pupae of *Apertochrysa* sp. stored under various periods of storage at different storage temperatures indicate high significant differences between the percentages of mortality. The highest mortality and highly significant differences with others mortality was recorded on pupae stored 30 days (same mortality on pupae stored 30-60 days) at 10°C (100%) followed by 62.99%, 47% mortality of pupae stored 40 days and 30 days at 15°C respectively. Whereas no significant differences were noticed in mortality of pupae stored 20 days at 10°C (32.22%) compared with nonstored pupae at 25°C (30.7%).

The lowest percentage of mortality 5.89% was recorded on pupae stored 10 days at 10°C but with no significant differences of mortality of pupae stored 10 days at 15°C (9.24%), 10 days at 20°C (20.59%) and 20 days (16.23%) at 20°C. (Table 3 A).

The highest percentage of survival of pupae stored of *Apertochrysa* sp. was recorded after 10 days storage at 10°C (94.1%) but it's not differ significantly with survival of pupae stored 10 days at 15°C (90.76%) and 20 days at 20°C (83.77%). No significant differences were noticed between the survivals of pupae stored 10 days at 15°C, 10 days (79.40%) and 20 days at 20°C.

The survivals of pupae stored 20 days at 10°C (67.78%), 20days (71.77%) and 30 days (53 %) at 15°C were showed to have no significant differences between them and in comparison with survival of nonstored pupae (69.30 %) at 25°C (Table 3 B).

The survivals rates of pupae stored were negatively affected and decreased linearly with the storage duration at 10°C and 15°C while positively increased at 20°C (for 10°C, y = -2.327x + 92.77, R^2 = 0.716, for 15°C, y = - 1-023x + 84.83, R^2 = 0.633, and for 20°C, y = 0.723x +70.25 , R^2 = 0.950) (Fig. 1). The lowest mortality was noticed at 10°C after 10 days storage while the highest mortality was recorded on pupae stored 30 days at 10°C. Lethal effects were noticed at 10°C related to the increasing of period storage. The mortality of pupae stored were increased linearly with storage period except in case of pupae stored 20 days at 20°C (for 10°C, y = 2.327x + 7.22, R^2 = 0.716, for 15°C, y = 1.023x + 15.6, R^2 = 0.633, and for 20°C, y = - 0.723x + 29.71, R^2 = 0.947) (Fig. 2). Similar results of survival were also showed in case of rearing the green lacewings *M. desjardinsi* (75% at 15°C to 91.9% at 22.5°C) and *Ch. nipponensis* (80% at 15°C to 95.2% at 22.5°C) (Nakahira *et al.*, 2005). Similar results were collected by Luczynski *et al.*, (2007) for storage of pupae of *Encarsia formosa* and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae).

Table 3. The mean ± SD of percentage of mortality, successful adults, disability, final number of active adults and pre-emergence period of pupa stored of *Apertochrysa* sp. at different temperatures and different storage periods.

Temperature	Percentage of mortality ±SD A	Percentage of successful adults ± SD B	Percentage of disability ± SD C	Pre –emergence period ±SD D	Percentage of high quality adults ± SD E
10°C/10days	5.89 ± 5.24 (33) f	94.1 ± 5.20 (33) a	9.26 ± 8.48 (31) d	9.60 ± 0.0 (31) b	85.39 ±12.53 a
10°C/20days	32.22 ± 1.92 (37) d	67.78 ± 1.92 (37) dc	43.45± 6.30 (25) b	13.20±0.14 (25) a	38.33 ± 4.41 cde
10°C/30days	100 ± 0.00 (35) a	0.00 ± 0.00 (35) f	-	-	0.00 ± 0.00 e
15°C/10days	9.24 ± 5.30 (54) f	90.76 ± 5.30 (54) ab	9.86 ± 4.60 (49) d	7.80 ± 0.13 (49) c	81.81± 2.42 ab
15°C/20days	28.23 ± 4.20 (90) de	71.77 ± 4.20 (90) dc	25.41± 4.40 (67) c	5.00 ± 0.17 (67) d	53.53 ± 6.32 bcd
15°C/30days	47.0 ± 3.14 (47) c	53.00 ± 3.14 (47) de	55.18± 5.00 (25) b	4.20 ± 0.41 (25) d	23.73 ± 2.28 de
15°C/40days	62.99 ± 5.55 (47) b	37.00 ± 5.55 (47) e	100.0 ± 0.00 (17) a	7.86 ± 0.15 (17) c	0.00 ± 0.00 e
20°C/10days	20.59 ± 1.91 (58) def	79.40 ± 1.91 (58) bc	17.32± 0.56 (46) cd	4.70 ± 0.06 (46) d	65.66 ± 1.75 bc
20°C/20days	16.23 ± 0.74 (43) ef	83.77 ± 0.74 (43) abc	19.39± 1.00 (36) cd	1.93 ± 0.23 (36) e	67.52 ± 1.48 abc
25°C	30.7 ± 10.62 (143) de	69.30±10.62 (143) dc	7.601± 1.28 (95) d	12.56±0.05 (95) a	64.00 ± 8.85 bc

Within the same column, the data followed by the same letter are not significantly different by ANOVA procedure on *P* = 0.05, (d.f = 18, P = 10, F = 168.21, M.S.D = 0.157)^A, (d.f = 18, P = 10, F = 29.82, M.S.D = 0.316)^B, (d.f = 16, P = 9, F= 224.76, M.S.D = 0.142)^C, (d.f = 18, P = 9, F= 1253.83, L.S.R = 1.562)^D, (d.f = 18, P = 10, F = 19.7, M.S.D = 0.395)^E. Column A, B, C, E data arcsine square root transformed before analysis.

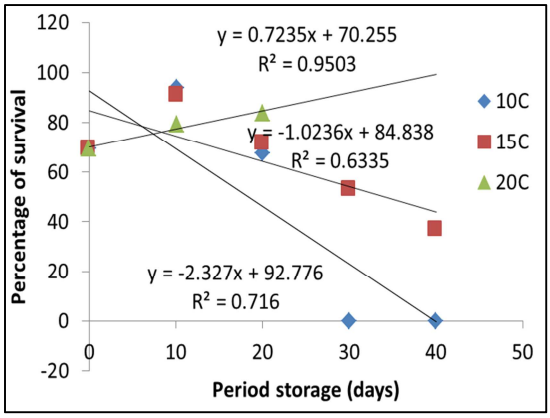

Fig. 1. The percentage of survival related to stored pupa undervarious periods of storage at different temperatures.

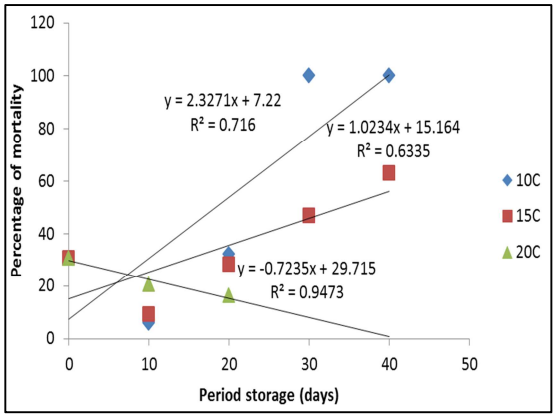

Fig. 2. The percentage of mortality related to pupa stored under various periods at different temperatures.

The disability of pupae stored

The results in (Table 3) C indicate that the percentages of disability of adults emerged from pupae of *Apertochrysa* sp. stored during different periods at 10°C, 15°C and 20°C were significantly different comparing with nonstored pupae. Storage the pupae 40 days at 15°C resulted in the highest disability (100%), which recorded highly significant differences with other storage periods and nonstored pupae. The disability of adults emerged from pupae stored 30 days at 15°C (55.18%), 20 days at 10°C (43.45%) and then 20 days at 15°C (25.41%) came second.

While the lowest disability of adults was recorded by nonstored pupae at 25°C (7.6 %) and with no significant differences with disability of adults emerged from pupae stored 10 days at 10°C (9.26%), 15°C (9.86%), 20°C (17.32%) and 20 days at 20°C (19.39%). The disability of adults emerged from pupae stored for various storage durations at different temperatures increased significantly and linearly with duration of storage period of pupae (for 10°C, $y = 1.792x + 2.178$, $R^2 = 0.784$, for 15°C, $y = 2.301x - 6.414$, $R^2 = 0.882$, and for 20°C, $y = 0.589x + 8.875$, $R^2 = 0.876$) (Fig. 3).

The disability range between 9.26 % after 10 days storage of pupae at 10°C to 100% after 40 days pupal storage at 15°C. This research could not find any previous attempts to determine the disability of pupae of green lacewing *Apertochrysa* sp., which makes this result an original contribution in this field.

Pre-emergence periods

The longest pre-emergence period 13.20 days was recorded under 20 days pupal storage at 10°C without significant differences with nonstored pupae 12.56 days at 25°C but significantly differed with pupae stored 10 days at 10°C (9.6 days). Under 15°C the pre-emergence periods decreased gradually with increasing the storage period which are 7.8 days, 5 days and 4.2 days after 10 days, 20 days and 30 days storage period.

While the pre-emergence period increased again in case of pupae stored 40 days at 15°C without significant differences with 10 days storage period at 15°C. The lowest pre-emergence period 1.93 days was noticed under 20 days pupal storage at 20°C (Table 3 D). The pre-emergence periods of adults from pupae stored at 15°C and 20°C were shortened gradually with increasing the temperature and increasing the duration of storage periods with significant differences comparing with nonstored pupae (for 15°C, $y = -0.277x + 11.53$, $R^2 = 0.909$, for 20°C, $y = -0.528x + 11.66$, $R^2 = 0.929$) (Fig. 4). While the pre-emergence periods of pupae stored at 10°C increased with increasing the storage periods ($y = 0.035x + 11.41$, $R^2 = 0.033$), it is attributed to prevent the development during pupal period at 10°C.

Slight development occurred at temperatures above 15°C and it is more clearly with pre-emergence period of adults emerged from pupae stored 20 days at 20°C (only 1.93 days).

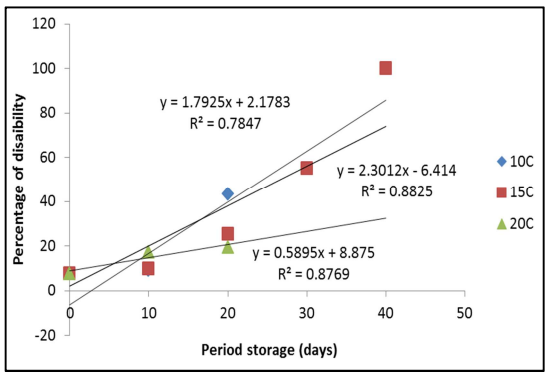

Fig. 3. The percentage of disability related pupa stored under various periods of storage at different temperatures.

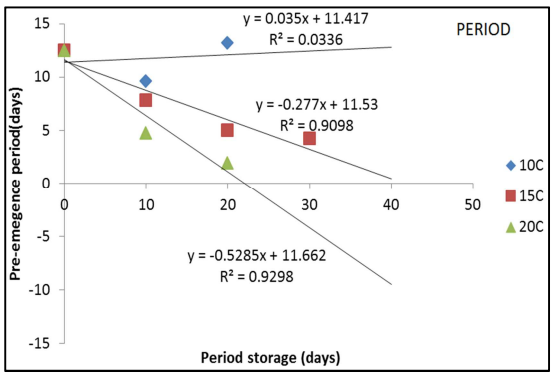

Fig. 4. The pre-emergence period of pupae stored related to various periods of storage at different temperatures.

However the storage of pupae 40 days at 20°C increased the pre-emergence period again because of the abnormal developments and negative effects on metamorphosis under this period which leads also to 100% disability. Similar results were recorded by Pandey and Johnson, (2005) during the storage of *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae). Also the same by Lopez and Botto, (2005) with the *Eretmocerus corni* and *E. Formosa* (Hymenoptera: Aphelinidae).

The final high quality adults

The best number of good adults produced was recorded under 10 days pupal storage at 10°C (85.39%) with no significant differences with the adults produced under pupal storage of 10 days at 15°C (81.81%) and 20 days at 20°C (67.52%). The second level of adults produced was noticed under pupal storage of 10 days at 20°C (65.66%) and 20 days at 15°C (53.53%) without significant differences compared with non stored pupae at 25°C. The percentage of adults produced from pupae stored 20 days at 10°C and 30 days at 15°C are 38.33% and 23.73 % respectively. However no adults were produced from pupae stored 30 days at 10°C and 40 days at 15°C (Table 3 E).

The numbers of good quality adults produced from pupae stored at different temperatures and various storage periods were affected negatively with increasing the storage period except in case of pupae stored at 20C (for 10°C, y = - 2.387x + 82.71, R^2 = 0.704, for 15°C, y = - 1.858x + 81.77, R^2 = 0.810, and for 20°C, y = 0.181x + 63.88, R^2 = 0.999) (Fig. 5). Zero percent of good adults was recorded from pupae stored at the longest period (40 days) at 15°C. While the high percentages of good quality adults were obtained from pupae stored short storage period (10 days) at 10°C (85.39 %) and 15°C (81.81 %). However the percentage of good quality of adults obtained from pupae stored 20 days at 20°C (67.52 %) did not differ significantly with highest percentages. Generally the high survival of pupae storage can be obtained from short storage periods (Lopez and Botto, 2005; Pandey and Johnson, 2005).

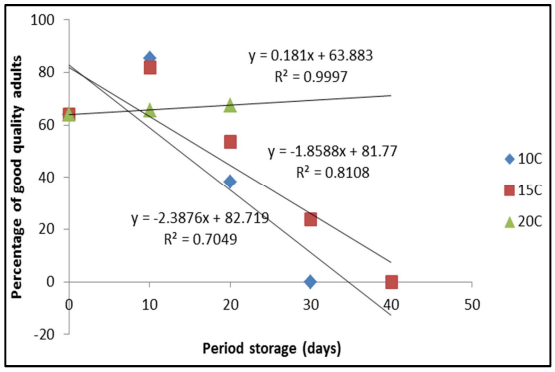

Fig. 5. The percentage good quality adults produced from pupae stored related to various periods of storage at different temperatures.

Conclusions

The developmental periods with and within the stages of *Apertochrysa* sp. were highly affected by the temperature. Among the temperatures tested the 25°C was the most suitable for development with best survival and shortest developmental period of *Apertochrysa* sp. The long developmental time at 15°C, 20°C and non development at 10°C give a good chance for storage the *Apertochrysa* sp. especially the pupae stage. Limited opportunities for storage of the eggs of *Apertochrysa* sp. at 15°C and 20°C (no more 7 days) because of the embryonic development during the storage period with high survivals of eggs and no diapause was happened after storage.

No any opportunities for storage the eggs of *Apertochrysa* sp. at 10°C because all the eggs were killed during the storage. The pupae of *Apertochrysa* sp. can be stored for brief period (10 days) at 10°C and 15°C because prolonged pupae storage is lethal. While the pupae of *Apertochrysa* sp. can be stored for short time (maximum 20 days) at 20°C because of the metamorphosis changing during the storage period at this temperature with high quality adults which are not differ significantly with adults produced from pupae stored 10 days at10°C, 15°C and nonstored pupae. More studies of thermal effects, on the size of adults, fecundity, fertility, lower and upper threshold temperature and degree-days (thermal constant K) and more studies of the effects of storage on the eggs and pupae of *Apertochrysa* sp. and after storage effects between 10°C to 15°C are needed.

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