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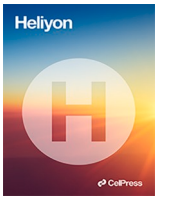

Research article

Effect of chilling on pupal developmental arrest and subsequent impact on quality control parameters of adult blowfly, *Lucilia cuprina* (Diptera: Calliphoridae)

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ABSTRACT

The chilling process will surely be a very cost-effective measure for repeated mass rearing and be an effective source for the requirements of a huge number of pupae collected and supplied for the sterile insect technique (SIT) program. In Bangladesh, blowfly (*Lucilia cuprina*) is a main cause of damage of marine fish during sun drying in coastal areas. The chilling for a certain duration at a certain temperature may store pupae for a specific time by reducing their metabolism. In this study, the effect of chilling temperature and exposure period on pupal developmental arrest was assessed and the subsequent impact on quality control parameters of the adult blowfly, *L. cuprina was also observed*. We considered the effects of three temperature (4, 7, and 10 °C) regimes and three exposure periods (10, 20, and 30 days) on 3-day-old pupae. Results showed that all the parameters were affected significantly by all the chilling temperatures and durations. Adult emergence, flight ability, pupal weight, adult longevity, fecundity, and fertility decreased with the increase of exposure duration, but partial emergence and pupal duration increased with exposure durations. Storages at 4 °C for 20 and 30 days had no adult emergence. Emergence occurred before the chilling duration at 10 °C for 20 and 30 days which clearly indicated the unsuitable condition for storage parameters. Though the emergence, flight ability, pupal weight, and longevity at 10 °C for 10 days had no significant difference with control, the fecundity and fertility did have a significant difference. In addition, treatment at 7 °C for 10days, the adult emergence, flight ability, pupal weight, longevity, fecundity, fertility, pre-oviposition, and oviposition period had no significant difference with control. Considering all the parameters, it can be concluded that the pupae of *L. cuprina* can be stored at 7 °C of chilling temperature for 10 days long without any deterioration towards the quality of adult flies.

1. Introduction

Chilling is a potential method to delay the development of insect life cycle, and efficient use to survive insects in adverse environmental conditions, and is useful for pest control strategies entailing mass rearing and release, such as biological control and Sterile Insect Technique (SIT) [1]. Without repeating mass rearing, storage of pupae at low temperatures is cost-effective and time-saving [2]. Therefore, the development of the chilling technique allows synchronization between available standardized stocks and wild flies for

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field releasing during the critical stages of pest outbreaks [3–5]. Especially, this method is working to improve the balance between productions of pupae and field releasing of adult flies by delaying the development period of pupae with minimal quality reduction [6].

Furthermore, the major obstacle to the successful implementation of mass releases is the availability of a huge number of flies and the cost of their rearing at the appropriate time [7]. Also, at present, many European countries export and import, insect pests and parasitoids as biological control and enable them to store for the duration as immature stage (pupae) and supply with required demand [8]. Overall, chilling or cold storage is developed for long-term storage and improves mass-rearing for sterile insect release programs [9]. However, the effect of chilling on the biology of blowflies was strongly observed.

Blowfly, *Lucilia cuprina* (Diptera: Calliphoridae) is regarded as the most destructive and dominant pest in the fish drying industry in Bangladesh [10]. Its other name is the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) which initiates more than 85% of fly strikes (cutaneous myiasis) on sheep in Australia [11]. Over 25% of marine fish are lost of the product during the process of sundering [12]. In the case of Sonadia Island (Southern coastal fish drying area), this loss could be up to one million U.S. dollars per year [13]. The farmers usually dip the raw fish into an insecticide solution just before sun-drying as a preventive measure against blowfly infestation. As a result, these insecticides poured against fishes create a greater hazard to public health as well as to nature [13]. To develop an alternative control measure, we tried to develop SIT against blowfly for adequate suppression of the blowfly population from coastal drying yards of Bangladesh.

The embryos of *Lucilia sericata* were stored as cryopreservation for a long time (8 years), and the percentages of hatching and larvae pupariated were not affected [14]. To examine temperature tolerance, the housefly pupae were stored at 7 and 10 °C and after 10 days, 85% of adult flies were emerged [15]. Adult emergence, pupal weight, adult flight ability, and fecundity decreased with the increase of chilling duration, but storage for a short duration (5 days) at 5 °C was not affected by mentioned parameters [16,17]. The pupae of *Lucilia sericata* can be stored at 4 °C for one year without uninterrupted rearing [18]. Queensland fruit fly pupae were highly sensitive to the lowest tested temperatures (13 and 15 °C), when the pupae were stored at 17 °C, pupal development time was more than doubled and only chilling temperatures above 19 °C did not significantly affect the percentage of fliers [6].Regarding the effect of chilling, though, there are several works on the development of larvae and pupae of *Lucilia cuprina* at different temperatures and durations [19–23]. But other biological parameters of adult flies after chilling durations of the pupae were not done. For other species of blowfly, *Lucilia sericata* [24,25], *Phormia regina* [26], the various effects of temperature on life history were observed. Also, the different techniques of preservation were studied for other blowflies [9,14,18]. Hence, more studies are needed to get appropriate biological activities of adult flies after the chilling duration of *Lucilia cuprina* pupae.

The objectives of our study were to (1) determine the effect of chilling temperatures and periods on quality control parameters of adult blowfly: adult emergence, flight ability, fecundity, fertility, and longevity, (2) find out a compatible chilling temperature and duration for pupae storage, (3) evaluate the impact of chilling on pupal developmental arrest, (4) retain the female flies as stock rearing which reduce the cost and time, and (5) delay the development time of *Lucilia cuprina* pupae with minimal quality reduction.

2. Materials and methods

2.1. Pupae

The experiment was designed by 3rd-day pupae (this age of *Lucilia cuprina* pupae is appropriate for chilling and sterilization). The pupae were collected and reared in the Blowfly lab (Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission). Stock rearing of *L. cuprina* was maintained at this lab. When blowfly obtained sexual maturity [27], the bovine liver was provided for ovarian development with a dish in stock cages, and from the 3rd day of ovarian development, eggs were collected by suppling tilapia fish (*Oreochromis niloticus*) with a dish in stock cages. The female laid eggs in mass or cluster. Eggs were gently separated from the fish substrate with the help of soft camel brush and forceps. It has been estimated that 1 g egg contains about 12,500 individual eggs. These eggs were used for rearing. Larvae were reared in stainless steel trays (52 × 47 × 6.5 cm) into which 4 kg raw tilapia fish (*Oreochromis niloticus*) per tray was supplied as larval diet. 2.5 g eggs were supplied into each rearing tray that was placed inside a stainless steel trays (67 × 53 × 16 cm) that contained 4 cm sawdust. After completing 3rd instar of larvae, they drop into sawdust and 3rd old pupae were collected by sifting. All research observations (emergence, flight ability, fecundity, fertility, longevity, pre-oviposition, and oviposition duration) were maintained under the same environmental conditions (27 ± 2 °C, 75 ± 5% RH, and 12:12 light/dark photoperiod).

2.2. Chilling temperatures and durations

The pupae were chilled at 4°, 7°, and 10 °C for 10, 20, and 30 days in cooled incubators (Model: Vs-3250BiPC-L). The relative humidity levels in the cooled incubator were 35–39%. Each batch was set with 5 replications and control. Three cohorts were used to evaluate the impact of chilling on biological parameters. Each replication consisted of 20 pupae that were placed in a small Petri dish (diameter 5.8 cm) for treatment in the incubator. After a particular duration, treated pupae were taken out from incubators and placed in cages (height 16 cm × width 13 cm × length 16 cm) supplying sugar in petri dishes and water with cotton that was socked into the conical flask (100 ml) as adult flies meal, and observe pupal period, emergence, flight ability, and longevity.

2.3. Pupal duration, emergence (%), and flight ability (%)

For the pupal duration, treated pupae were observed daily until emergence. For inspection flight ability of treated pupae that were

set in PVC flight tubes (radius 4.5 cm and height 10 cm) that were wiped off by talcum powder to provide an additional vertical surface (to prevent newly emerged flies on which to rest) and were placed on a Petri dish (diameter 9.2 cm) and set in cages that were rounded with mosquito net. After emerging, adult flight and flightless fly were counted on the base of flight ability.

When all emergences had ceased (3 or 4 days after the first flies emerged), the remaining contents of the tubes were counted. Following FAO/IAEA/USDA [28]; individual flies were classified as (1) not emerged (adults within the unopened pupal cases); (2) partially emerged (adults stuck in pupae, but partially outside); (3) deformed (if they had completely shed the pupal case but had damaged wings); (4) not fliers (if they had completely shed the pupal cases and had morphologically normal wings, but failed to escape the tube), and (5) fliers (the number of flies that escaped the tube).

Then for the standard assessment of data, the required data were included in the emergence and flight ability assessment form and calculated percentages of emergence and flight ability. Following FAO/IAEA/USDA [28], the calculation formula of the percentage of emergence; $T - (A + B)/T^*100$, the percentages of flight ability: $(T - (A + B + C + D))/T^*100$ (Here, T = Number of pupae, A = Not emerged, B = Partially emerged, C = Deformed, D = Not fliers).

2.4. Fecundity, fertility, pre-oviposition, and oviposition duration

Female fecundity and egg hatchability were estimated from treated pupae (three temperatures and durations). For estimated fecundity, fertility, pre-oviposition, and oviposition duration, male and female flies were separated from newly emerged flight able flies. For experiment fecundity and fertility, we assemble one males and one females per cage (16 cm × 16 cm × 13 cm), and supplied sugar and water as an adult meal. There were five replications for each treated flies (combination of temperature and time). From 3days after emergence, when blowfly obtained sexual maturity [27], 5 g to 10 g of the bovine liver was provided for ovarian development with a Petri dish in each cage, and within 24 h, the bovine liver was removed. On the second day, after providing bovine blood, a Petri dish containing 30 to 50 g pieces of fish was supplied in each cage every second day and the lights of the experimental room were put out. Within 3 h, the pieces of fish were removed and assessed for eggs laid to determine female fecundity. If eggs were present, they were carefully collected and weighed to determine the approximate number of eggs, and then placed on an artificial diet of the larvae of this species where they were allowed to hatch. Recording fecundity and fertility continued until up to 80% of females had died. From the fecundity and fertility cages, we observed pre-oviposition and oviposition duration.

2.5. Longevity

After collecting the data of emergence (%) and flight ability (%), flies were reared in cages by supplying water in soaked cotton wool that was inserted in a 100 ml conical flask and sugar in a Petri dish as a meal. Each cage was set up with 20 males and females in a ratio of 1:1 with five replications. Daily each cage was checked for longevity and supplied meals if needed. Dead flies were removed and recorded daily along with their gender.

2.6. Data analysis

The data of affected variables (emergence, partial emergence, pupal weight, pupal duration, flight ability, longevity, fecundity, fertility, pre-oviposition and oviposition period) and treatment variables (temperature and duration) were analyzed using two-way analysis of variance (ANOVA) with general linear model (GLM). Before the parametric test, all variable data were checked using

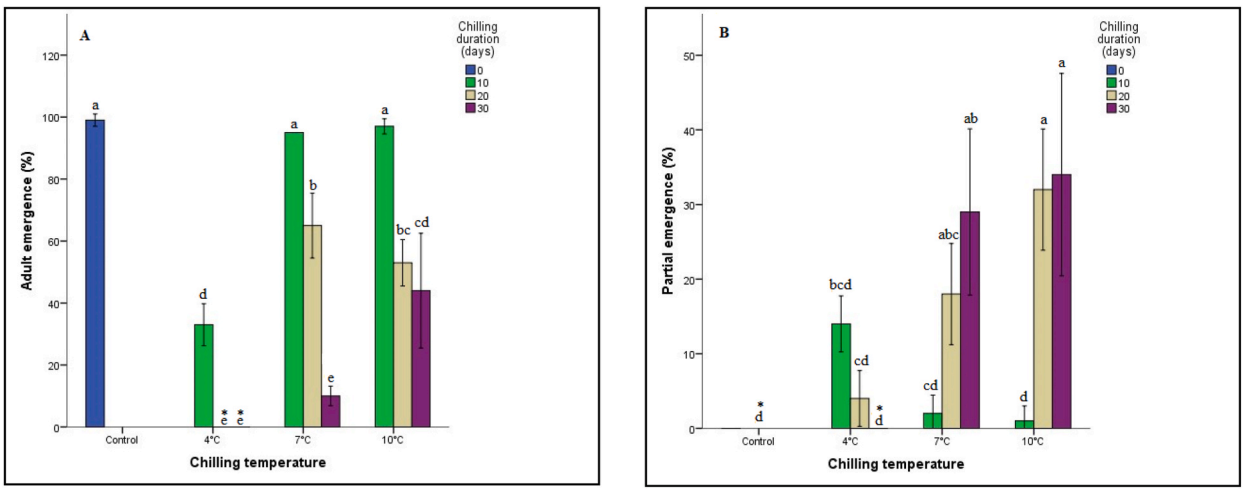

Fig. 1. Mean (±SE) percentages of adult emergence (A) and partial emergence (B) of *Lucilia cuprina*, chilling at different temperatures and durations. Different letters above the histogram bars indicate significant differences (Tukey test: $P < 0.05$). *Data value of these bars is zero.

normality (Shapiro-Wilk) test. Then, non-normal percentage variables were transformed into arcsine square root values to decrease the error values, assume normal distribution and homogeneity of variance before ANOVA test [29]. In these analyses, temperature and duration were inputted as independent variables and all biological parameters were inputted as dependent variables. Also, Tukey's multiple comparison test was done to compare significant differences among different temperatures and durations of *Lucilia cuprina*. The significant level was set at $P < 0.05$. All data were analyzed using statistical software IBM SPSS V. 21.

3. Results

3.1. The adult emergence and partial emergence

The percentages of emergence were affected significantly by the chilling temperature ($F_{2,40} = 254.067$, $P < 0.001$) and duration ($F_{2,40} = 227.804$, $P < 0.001$), and the interaction of chilling temperature × duration was also significant ($F_{4,40} = 13.820$, $P < 0.001$). Similarly, the partial emergence differed significantly among chilling temperature ($F_{2,40} = 20.489$, $P < 0.001$) and duration ($F_{2,40} = 21.127$, $P < 0.001$) and also, the interaction of chilling temperature × duration was significant ($F_{4,40} = 32.661$, $P < 0.001$).

In a post hoc comparison using the Tukey HSD test, chilling of pupae for 10 days at 7° and 10 °C had no significant effect on their emergence ($P > 0.05$) but 4 °C had significantly decreased with control ($p < 0.05$) (Fig. 1A). Similarly, the partial emergence at 7 °C for 30 days, and 10 °C for 20 and 30 days had a significant difference with the control, but 10 days (7 °C, 10 °C) showed no significance (Fig. 1B).

3.2. Pupal duration and pupal weight

The results of pupal durations were influenced statistically by the chilling temperature ($F_{2,40} = 186.411$, $P < 0.001$), chilling duration ($F_{2,40} = 1089.055$, $P < 0.001$), and the interaction of chilling temperature × duration ($F_{4,40} = 80.541$, $P < 0.001$). Similarly, after being treated, the pupal weight was varied significantly by the chilling temperature ($F_{2,40} = 85.664$, $P < 0.001$), chilling duration ($F_{2,40} = 164.623$, $P < 0.001$), and the interaction of chilling temperature × duration ($F_{4,40} = 27.520$, $P < 0.001$).

With increased chilling duration, the pupal duration (pupal development time) increased. Some adult flies emerged in incubator before storages duration at 10 °C for 20 and 30 days.

A long chilling duration of more than 10 days declined the pupal weight. The pupal weight for 20 and 30 days at 7° and 10 °C, and also 30 days at 4 °C were significantly lighter than the control ($P < 0.05$), but for 10 days at 4° and 7 °C had no significant difference ($P > 0.05$) (Fig. 2B).

3.3. Adult flight ability and longevity

The percentage of adult flight depended significantly on chilling temperature ($F_{2,40} = 127.723$, $P < 0.001$), duration ($F_{2,40} = 311.297$, $P < 0.001$); and also, the interaction of chilling temperature × duration had a significant effect ($F_{4,40} = 35.981$, $P < 0.001$). Similarly, The longevity differed significantly among chilling temperature (Male: $F_{2,40} = 24.998$, $P < 0.001$; Female: $F_{2,40} = 17.768$, $P < 0.001$) and duration (Male: $F_{2,40} = 158.113$, $P < 0.001$; Female: $F_{2,40} = 81.085$, $P < 0.001$); and also, the interaction of chilling temperature × duration had significant effect (Male: $F_{4,40} = 9.386$, $P < 0.001$; Female: $F_{4,40} = 14.508$, $P < 0.001$).

From the pupae at 7° and 10 °C for 10 days, above 90% of flies were flight after emergence that had no significant difference with

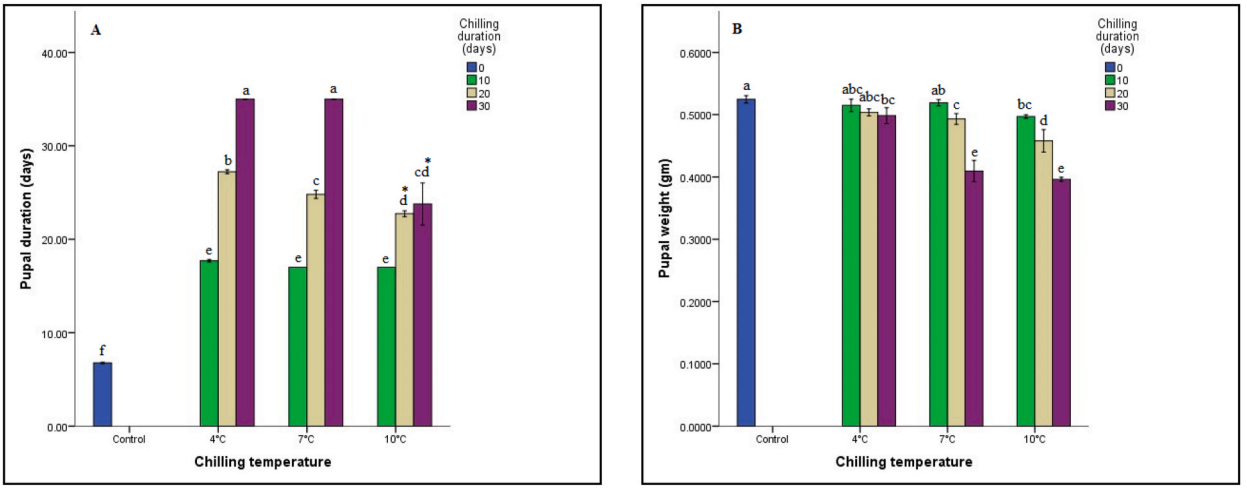

Fig. 2. Mean (±SE) pupal duration (A) and pupal weight (B) of *Lucilia cuprina*, chilling at different temperatures and durations. Different letters above the histogram bars indicate significant differences (Tukey test: $P < 0.05$). *Some adults emerged before chilling duration.

control (P > 0.05). °C With the increase of chilling duration of more than 10 days, the flight ability declined significantly (P < 0.05) (Fig. 3A).

Only the adult longevity of females at 7 °C for 10 days had no significant difference with control (P > 0.05). Again, the longevity (female) at 4° and 10 °C for 10 days decreased significantly than the control (P < 0.05) (Fig. 3C). Similarly, the longevity (male) at 7 °C for 10 days had no significant difference with control (P > 0.05) but at 4° and 10 °C for 10 days had significantly shorter survival than control (Fig. 3B).

3.4. Fecundity and fertility

The fecundities of flies were significantly different among temperature ($F_{2,40} = 4.047$, P = 0.025) and duration ($F_{2,40} = 5.219$, P = 0.01); and also, the interaction of these two independent factors was significant ($F_{4,40} = 4.031$, P = 0.008). Similarly, fertility was affected significantly by temperature ($F_{2,40} = 5.762$, P = 0.006) but chilling duration ($F_{2,40} = 2.964$, P = 0.063) had no significant effect and the interaction of two independent variables had a little significant ($F_{4,40} = 3.484$, P = 0.016).

The mean fecundity was highest for 10 days at 7 °C but had no significant difference with control (P > 0.05). Though, the fecundity was highest for 10 days at 7 °C but the fertility was less than control and showed no significant difference (P < 0.05). Otherwise, the fertility for 20 days at 7° and 10 °C had no significant difference with 7 °C for 10 days (P > 0.05) but significant difference with control (P < 0.05) (Table 1).

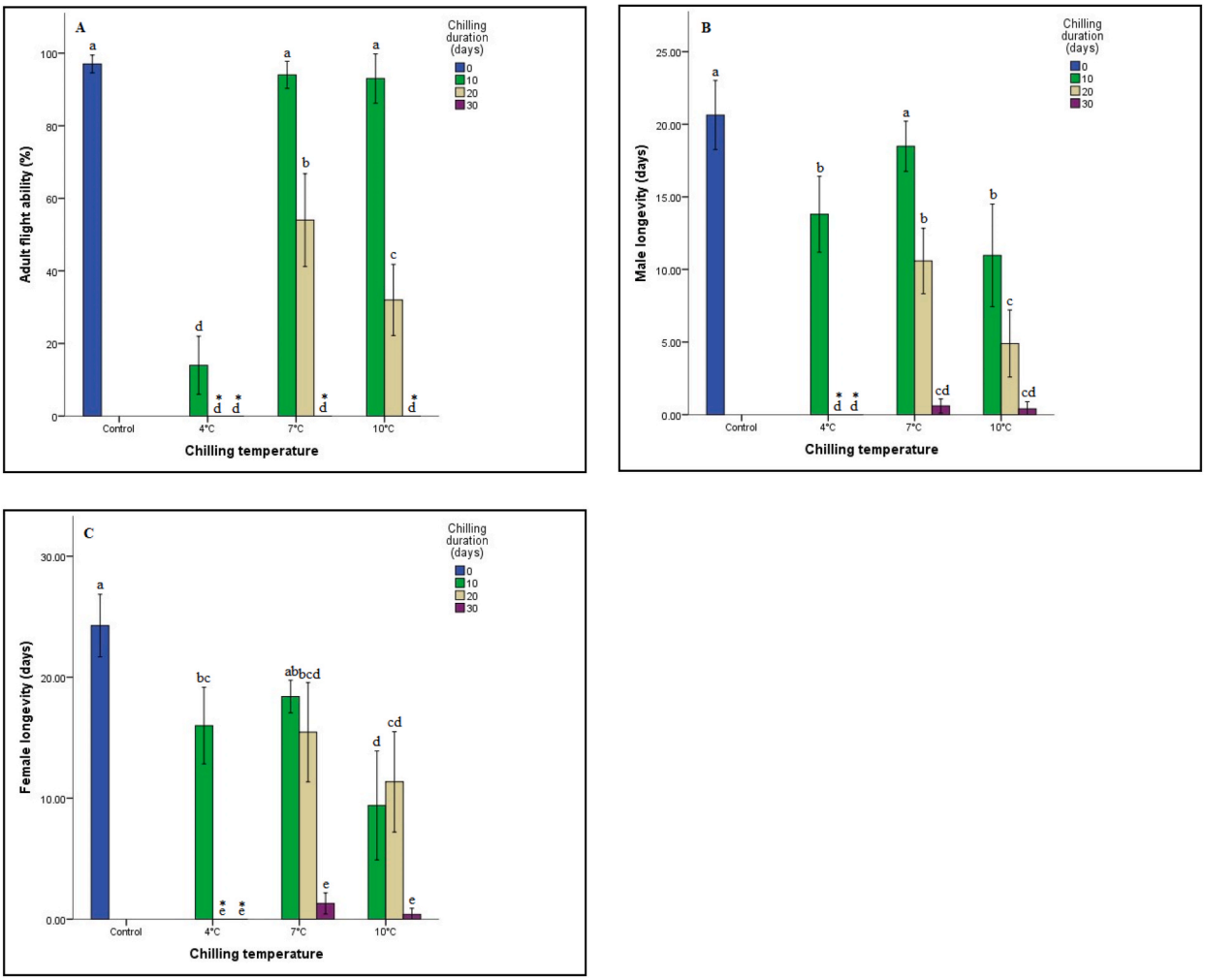

Fig. 3. Mean (±SE) percentages adult flight ability (A); mean (±SE) male (B) and female (C) longevity (days) of *Lucilia cuprina*, chilling at different temperatures and durations. Different letters above the histogram bars indicate significant differences (Tukey test: P < 0.05). *Data value of these bars is zero.

3.5. Pre-oviposition and oviposition duration

Pre-oviposition duration varied a small significant among exposed temperature ($F_{2,40} = 4.710$, $P = 0.015$) and duration ($F_{2,40} = 3.349$, $P = 0.045$) but the interaction of chilling temperature × duration had no significant effect on pre-oviposition period ($F_{4.40} = 2.344$, $P = 0.071$). Similarly, the chilling temperature ($F_{2,40} = 1.176$, $P = 0.319$), duration ($F_{2,40} = 1.176$, $P = 0.319$) and the interaction of temperature × duration ($F_{4,40} = 1.119$, $P = 0.361$) had no significant effect on oviposition duration.

The longest pre-oviposition duration was reported for 10 days at 7 °C but no significant difference with control ($P > 0.05$). The mean oviposition duration for 10 days at 7 °C was recorded as 6.4 days which had no significant difference with control ($P > 0.05$). Though, the oviposition duration was comparatively short for 20 days at 7° and 10 °C but so significant difference with control ($P > 0.05$) (Table 1).

4. Discussion

4.1. The adult emergence and partial emergence

There was no emergence at 4 °C for 20 and 30 days, and the emergence declined 50% at 7° and 10 °C more than 20 days as duration. Benelli et al. [6] observed that when Queensland fruit fly pupae were stored at 13° and 15 °C, the emergence rate was zero, also at 17 °C the emergence rate was almost 50%. Similar results were supported by the work of Diallo et al. [30] that observed the long, and low-temperature periods affected significantly and reduced the emerging rate. °C Again, This result is in contrast with previous studies of the low-temperature effect on *Glossina palpalis gambiensis* by Mutika et al. [31] that showed the emergence of pupae after chilling at low temperature (10° and 12.5 °C) for 3, 5, or 7 days was similar to those kept under standard colony conditions.

Our result reported that the partial emergence at low temperature (4°, 7 °C) was lower than 10 °C but the pupal mortality was higher at low temperature (4 °C). A previous study observed that the highest level of mortality was at the pupal stage which were the least cold tolerant [15,25].With increasing low-temperature duration, partial emergences were increased and the previous study [32, 33] suggested that, mortality (partial emergence) of pupae was affected by long-term chilling temperature. The data of our study indicate that the pupae of *Lucilia cuprina* can not be stored more than 10 days at 7° and 10 °C, because partial emergence above this duration was negatively affected.

4.2. Pupal duration and pupal weight

These results suggested that the embryonic development in pupae at low temperatures occurred slowly but not halted, so *L. cuprina* pupae could not be stored more than 10 days at 10 °C. Dallwitz [22] observed that the pupal development of *L. cuprina* was completed at constant 15 °C in 26–28days but not at 10 °C. In another work, results showed that development of the *L. cuprina* was slow at lower temperatures, but at the high temperature, the developmental rate was fast [19]. In another study, Johl and Anderson [34] observed similar results for other blowfly species, *Calliphora vicina* (Diptera: Calliphoridae), and obtained if the egg, larvae, and pupae were exposed for 24 h at an ambient temperature of 3 °C, The adult emergence was delayed 24 h, because the insects did not appear to develop while chilled. This study reported that the pupal weight had no difference for 10 days at three exposure temperature, but the weight declined with increased duration. Under chilling, the pupae of blowfly and other flies were dehydrated for low humidity at incubator. This report suggested that pupal weights with short duration are not affected significantly. Benelli et al. [6] highlighted that after chilling duration, the pupal weight was reduced at the low temperature with long-termduration, and the studies by Vargas et al. [35], Eskafi and Fernandez [36], and Hulthen and Clarke [37] reported that the weight loss of pupae is a consequence of water loss at chilling duration.

Table 1
Mean (±se) fecundity (number of eggs laid per female), fertility (%), pre-oviposition duration, and oviposition duration of *Lucilia cuprina,* chilling at different temperatures and durations. Values among the columns followed by different indicate significant differences (Tukey test: $P < 0.05$). *Data value is zero.

Treatment		Variables (mean ± SE)			
Temperature	chilling duration (days)	Fecundity	Fertility (%)	Pre-oviposition duration (days)	Oviposition duration (days)
Control	0	175.8 ± 50.95 ab	75.86 ± 19.014a	6.8 ± 2.92 ab	6.8 ± 4.53a
4°c°C	10	0.00b*	0.00c*	0.00b*	0.00a*
	20	0.00b*	0.00c*	0.00b*	0.00a*
	30	0.00b*	0.00c*	0.00b*	0.00a*
7 °C	10	240.8 ± 95.144a	54.41 ± 13.00 ab	11.8 ± 2.87a	6.4 ± 4.26a
	20	27.727 ± 12.40b	19 ± 19.00bc	4.2 ± 4.20 ab	0.2 ± .20a
	30	0.00b*	0.00c*	0.00b*	0.00a*
10 °C	10	36.2 ± 36.20b	0.00c*	2 ± 2.00 ab	0.2 ± .20a
	20	32 ± 32.00b	14 ± 14.00bc	3 ± 3.00 ab	0.2 ± .20a
	30	0.00b*	0.00c*	0.00b*	0.00a*

4.3. Adult flight ability and longevity

These results indicate that very low temperature and long chilling duration affected the morphological structure of flies and short-term duration of 7° and 10° had no effect on flight ability. Couillien and Gregoire [38] who suggested that the loss of reserved fat body during chilling duration could be a reason for the reduction of mobility in the beetle predator *Rhizophagus grandis*. Similar results were observed for the storage of mature pupae of *Glossina p. gambiensis*, *Glossina pallidipes* and *Haematobia irritans* at low temperature (7–10 °C) for 5 days (120 h), which had no detrimental effect on flight ability [31,39].

Since, the longevities of adult flies that emerged from 10 days at 7 °C were greater than 20 and 30 days, and also had no significant difference with control. These results are agreement with Yılmaz et al. [40] who observed the negative effect of chilling at 10 °C for 1–4 weeks duration on *Trichogramma evanescens* Westwood and reported that the longevity of adults declined with the increase of the chilling durations. The adult longevity declined, because the flies that emerged from low temperature and long duration were unable to fly, so they could not take adult food as sugar and water [41]. In our study, the longevity of males and females that emerged from the storage pupae at different temperatures for 30 days was worse affected. These findings are assent with Rundle et al. [42] who reported that chilling over three weeks of *T. carverae*, *T. nr. brassicae* and *T. funiculatum* carver had a negative effect on longevity. In another study, Boivin [43] explained that cold-induced poor health due to insufficient food reserve during their immature stage in pupae is a cause of negative effect on longevity.

4.4. Fecundity and fertility

Very low temperature (below 7 °C) and long duration (above 10 days) affected fecundity and fertility. These results were synchronized with the fecundity of *Hippodamia variegata* (Coleoptera: Coccinellidae) that was stored at different low temperatures and duration, and the fecundity was decreased with very low temperature and long duration [44]. Also, Jones and Kunz [39] reported that resulting immature ovaries of mature female and negative impact on mating success, the fecundity was affected by low temperature and no mature eggs were produced. The optimum chilling temperature and duration for *Lucilia cuprina* can be 7 °C and 10 days, because the fecundity and fertility were highest at these treated parameters. Also, many researchers have observed that the fecundity and fertility of females were decreased with increased chilling duration and low temperature, which adjourns metabolic maintenance at the immature stage, and halts fat reserve [32,45–47].

4.5. Pre-oviposition and oviposition duration

Pre-oviposition duration was higher at 7 °C for 10 days than control because the flies used more energy in chilling duration at low temperature, so more extra energy and time is required to mature ovary than control, but for 20 days and at 10 °C for 10 and 30 days showed lower pre-oviposition durations that were unexpected and erratic, because Jones and Kunz [39] and Ozgokce et al. [48] reported that the pre-oviposition durations were correlated positively with increasing chilling duration. In another study on ladybird beetle, its pre-oviposition duration was extended with prolonged chilling but interestingly the ladybird that experienced 90 days chilling at 0 °C had the shortest pre-oviposition duration [49]. Otherwise, Jones and Kunz [39] and Kondur [50] observed in their works, the oviposition periods were reduced significantly by the chilling duration when duration were prolonged above 3 weeks.

5. Conclusion

This study shows the chilling effect, which delayed the development time of *Lucilia cuprina* pupae with minimal quality reduction, and retain the female flies as stock rearing which reduce the cost and time. The pupae of this fly can be stored by chilling up to 10 days at 7 °C because this temperature and duration had no effect on quality control parameters. Though emergence, flight ability, longevity and pupal weight were not affected at 10 °C for 10 days fecundity and fertility were affected negatively. Further now, we are studying the effect of irradiation and chilling on pupae at different ages all at once.

Declarations

Author contribution statement

Md. Mosharraf Hosain: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Muhsina Yasmin: Conceived and designed the experiments; Performed the experiments.
Md. Shahinur Islam: Analyzed and interpreted the data; Wrote the paper.
A.T.M.F. Islam: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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