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COLD-PRESERVATION OF *Lucilia sericata* (DIPTERA: CALLIPHORIDAE) PUPAE AND ADULT PRODUCTS AS A NEW VENTURE TO ADULTS REARING

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KEYWORDS

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ABSTRACT

Rearing insect colonies in insectarium is often encountered with some problems. Cold-preservation can serve as an alternate option and can save large amount of insects without time-lapse, this method is also found valuable and cost-effective. In present study *Lucilia sericata* adults were used for cold preservation. One hundred twenty (120) *L. sericata* pupae were used in this study, these insects were divided in three groups of forty member, and each group was stored in 50 ml tubes containing sawdust and kept at 4°C for 3, 6 & 12 months. After the completion of required exposure time (3, 6 and 12 months), pupae were taken out from 4°C and placed under standard maggotarium conditions on sawdust in 40 × 40 cm cages. This was followed by the counting adult number, result of study revealed that after three months, 28 (70%) pupae were transformed into adults. While in case of 6 and 12 months this transformation number was 20 (50%) and 8 (20%) pupae respectively. From the result of study it can be concluded that this method is cost effective and time saving and *L. sericata* can be reared when ever required. This method opens new horizons for mass rearing of *L. sericata* for laboratory, research and business requirements

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1 Introduction

Rearing of insect colonies in insectaries is faced with various problems such as colony breakdown, diseases, genetic drift, high labor and material costs and finally time-consuming (Leopold et al., 2001; Augustinos et al., 2016). Choices to continuous culture include the various stages of insect life cycle which undergo diapauses (Bourdais et al., 2012; Lee, 2012; Rinehart et al., 2013; Rajamohan et al., 2014). Cryopreservation or cold preservation are the methods which can maintain insects for along time in laboratory application and mass production when needed (Leopold et al., 2001). These techniques were successfully established in many insects species including embryo of *Lucilia sericata* and *Bombyx mori* but most cold storage procedures still need experimental verification (Rajamohan et al., 2014). Accordingly, degree of maintenance, sub-optimized conditions and reliable viability can be expected for only a few months of storage (Rajamohan et al. 2014). Longer periods of storage can affect the viability of insects, so it needs specific technique which can increase the survival of insect without any effect on a particular stage.

Longer periods of storage can be achieved through cryopreservation, although till date only six dipteran and two lepidopteran species have been successfully cryopreserved (Rajamohan et al., 2013; Augustinos et al., 2016). Insect eco-physiological features played important role in tolerating cold weather or cold tolerance, this can be used to maintain insect under cold-preservation. With the start of the cold season, the insect save extra energy to overcome winter. During cold, several biochemical changes such as change in total glucose, lipid, protein, and glycogen levels occurred in the insect body, which help these to survive under cold conditions. Insect capacity to tolerate low temperatures is referred as cold hardiness or cold tolerance (Lee, 1991; Santacruz et al., 2017). During cryopreservation, insects may face chilling injury, in this ice crystal forms inside the insect's body which lead to mechanical and osmotic stress (Sheikh et al., 2017). Cold shock damage is caused by brief exposure to below zero degrees (Chidawanyika et al., 2017; Saeidi et al., 2017). Freezing resistant insects are not vulnerable to the formation of ice crystals in their tissues because they contain proteins or peptides that protect them against extracellular freezing (Horwath & Duman, 1983). However, in many species that show this physiological compatibility, survival depends on the temperature and the length of time spent in the frozen state. Also, most insects do not tolerate freezing (Salt, 1961).

The blow fly *Lucilia sericata* (Meigen 1826) (Diptera: Calliphoridae) (Beverley, 2012) is a fly with medicinal importance, it is also used in forensic medicine and agriculture (Tomberlin et al., 2017; Moemenbellah-Fard et al., 2018). From

ancient time, *L. sericata* larvae have been reportedly applied to treat necrotic, infected and non-heal injuries, such as bedsore, burn and diabetic foot ulcers (Sherman & Shimoda, 2004; Akbarzadeh et al., 2012). In medicine, larvae of *L. sericata* are used to treat wounds or applied in maggot debridement technology (Mirabzadeh et al., 2017). The larvae of *L. sericata* secrete antibacterial peptide, proteases and growth factors in wound which affect the wound healing process (Alipour et al., 2017a). Further, these larvae feed on necrotic tissues of wound and change the pH of wound from acid to alkaline which leads to stimulation of the repair process. Therefore, due to the risk of damage to vital organs, risk of infection in surgical operations, speed of treatment, recovery in patients and the limited use of antibiotics, maggot therapy is recommended as a selective therapeutic approach (Rafinejad et al., 2014; Alipour et al., 2016; Hajimohammad et al., 2017)

In general, the use of larvae on a wound is the last option of defense and it is usually used when a patient has undergone antibiotic and surgical treatment and these two are completely unsuccessful (Rafinejad et al., 2014; Naafs, 2017). In forensic medicine, it is an important marker species used in the survey of the time since death that named post mortem interval (PMI) (Zajac et al., 2018). These flies are very important insects in criminal studies because a part has been created in forensic medicine (Bajerlein et al., 2018).

Larvae are generally colored and legless, have a sharp, upright tip, and are equipped with respiratory tract, which is one of the reasons for inter-species diagnosis (Amendt, 2004; Moemenbellah-Fard et al., 2018). By considering its medicinal, forensic and agricultural importance, the expansion of cold-preservation methods for the long-term storage is very significant under laboratory conditions (Sheikh et al., 2017). Present study has been undertaken to estimate the important factors which determined the cryopreservation of *L. sericata*. Further, potential roles that pupae play under cold conditions are also studied in present study and listed in Tables 1 & 2.

2 Materials and methods

2.1 Rearing of *Lucilia sericata*

The *L. sericata* colony used in this study was established on flies that were collected from field of Shiraz city of Fars province. The insects used in this study were the Old World Screw worm and therapeutic myiasis agents used in treatment of necrotic wounds (Alipour et al., 2017b). Adults were exposed to a 12h light/dark cycle, at a relative humidity of 40–50%, and temperature range at 18–25°C under controlled conditions. The larvae were fed on ground chicken liver. Accurate species identification was confirmed by using morphological (Akbarzadeh et al., 2015) and

Table 1 Viable and non-viable pupae of *Lucilia sericata* after three periods of time under cold-preservation

Status	First period 3 months storage	Second period 6 months storage	Third period 12 months storage	Statistical analysis based on the time of storage in 4°C
viable	28(70%)	20(50%)	8(20%)	P<0.05
Non-viable	12(30%)	20(50%)	32(80%)	

Table 2 Comparison of three groups of *L. sericata* pupae after three periods of time under cold-preservation

Groups	Number of pupae per group	4°C Storage period	Time of Release in sectary conditions	Viable pupae		Statistical analysis between all groups
				Number	Percentage	
Groups 1	40	3 Month	After 48 hour	8	20	P<0.05
			After 96 hour	14	35	
			After 125 hour	6	15	
Groups 2	40	6 Month	After 48 hour	6	15	
			After 96 hour	11	27.5	
			After 125 hour	3	7.5	
Groups 3	40	12 Month	After 48 hour	2	5	
			After 96 hour	5	12.5	
			After 125 hour	1	2.5	

molecular tools (Tourle et al., 2009). Experiments were performed on the pupae of *L. sericata* fly from a colony that had been reared in Shiraz School of Health insectarium, Shiraz University of Medical Sciences (SUMS).

2.2 Long-Term Cold-preservation

One hundred twenty (120) *L. sericata* pupae were divided in three groups (40 in each group) and were stored at 4° C on 25 June 2016 and retrieved on 25 June 2017. The pupae were transferred to a 50 ml falcon tube and were placed in a refrigerator at 4°C. Hereafter, at the interval of 3, 6 and 12 month, 40 pupae were taken out from the refrigerator and placed under standard maggotarium conditions on sawdust in a 40 × 40 cm cage.

2.3 Pupae viability and Data analysis

The procedure described above was used to revive the pupae, and the viability percentages were recorded. The percentages of pupae viability were evaluated based on the number of adults alive. Furthermore, statistical analysis was done by one-way ANOVA method and chi-square test in SPSS V.21 program was applied to compare the groups.

3 Results

First group of pupae which were taken out from the refrigerator (from 4°C) after 3 months, out of 40 extracted pupae only 28 (70%) of them were developed in to adults. While in case of second group (after 6 months) only 20 (50%) and in third group (after 12 months) only 8 (20%) of them were transformed in to the adults (Table1). Result of study revealed that with the increasing storage time at 4°C viability of pupae were decreased and this difference between viability and time of storage at cold conditions are significantly different ($P<0.05$).

The first group that were stored at 4°C for 3 months, the pupae were placed in mentioned cages in the maggotarium and after 48 hours 8 pupae were matured , while after 96 hours 14 (35%) and after 125 hours 6 pupae were matured from 40 pupae. In second groups that were stored at 4°C for 6 months, after 48 hours, 6 pupae were matured while in case of 96 and 125 hours 11 and 3 pupae were matured respectively from 40 pupae . In third group that were stored at 4°C for 12 months, after 48 hours, 2 pupae were matured, after 96 hours 5 pupae and after 125 hours 1 pupae was matured from 40 pupae (2.5%) This study showed a significant difference between three groups ($P<0.05$) (Table 2).

4 Discussion

In this study viability of *L. sericata* pupae was assessed after storage at 4°C for 1 year. Survival was influenced by the length-time of the storage period. Studies have shown that insects have cold tolerance due to physiological and ecological adaptations (Overgaard & MacMillan, 2017; Sheikh et al., 2017). So far, many studies have been carried out on the storage of eggs in freezing conditions and nitrogen tanks (Rajamohan et al., 2014), but effect of cold storage on insect pupae, especially on the fly pupae of *L. sericata*, has not been studied yet. This study revealed that adult flies of *L. sericata* can be obtained by storing the pupae at 4°C for one year without continuous rearing under maggotarium conditions. Therefore, there is no need to continuous rearing and care of this insect in maggotarium for molecular and morphological tests. This method can help researchers to mass rear without spending extra time and costs. In addition, cold-preservation method can help maggot therapy clinics in treatment of burns or diabetic wound (Saleh et al., 2014).

Cold acclimation of the green bottle blowfly pupae, *L. sericata*, could be manipulated to implement wound debridement therapy in a tropical region where many infectious diseases also prevail (Azizi et al., 2012; Farhadpour et al., 2016; Neghab et al., 2006). In this study, first time cold preservation of 120 *L. sericata* pupae was carried out under laboratory conditions. Wang preserved using cryo-preservation method in house fly which later led to development of a protocol for screw worms (Wang et al., 2000). The results of this research has many common features with previous studies on insect species such as *M. domestica*, *C. hominivorax*, *Ceratitis capitata*, *Wiedemann* and *Anastrepha ludens* (Leopold, 2007; Handler et al., 2009). This protocol was refined and used for preserving 12 screwworm strains, about 25000 embryos (Leopold et al., 2001; Suszkiw, 2005). We deduce that the cold-preservation protocol can be prosperous altered to the rearing of *L. sericata* in laboratory. From the result of study, it can be concluded that cold-preservation method reported in present study will benefit the current and upcoming users of this fly for research in medical entomology field, forensic entomology and larva therapy in medicine to treatment of wound healing by reducing the costs connected with mass product fly in laboratory condition. Eventually, it was expected that the development of this protocol for another insect species will increase its approval as an important tool for those who work on rearing insects.

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Conflict of interest

All the authors declare that there is no conflict of interest.

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