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Effect of Sterilization on *Lucilia sericata* (Diptera: Calliphoridae) Eggs and larval stage in laboratory condition

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A B S T R A C T

Introduction: Maggot debridement therapy (also known as larval therapy) is a type of biotherapy that involves the use of live, sterile maggots (flies' larvae) to clean up non-healing and soft-tissue wounds, treat the necrotic (dead) and sloughy tissue. This method represents a controlled myiasis in which necrophagous fly larvae will clean the wound and stimulate growth hormones, cell reproduction, and the body's immune system. This group of flies, commonly known as necrophagous flies and have no harmful effects on healthy tissue, is the most appropriate choice. This research was conducted to investigate the effect of being sterile or non-sterile on the hatching rate of eggs and the persistence of the larval stage (1st instar) of *Lucilia sericata*.

Materials & Methods: A sweep net and bottle fly traps (reverse-cone model) were used to collect flies and identified by using valid systematic keys. The *L. sericata* eggs were placed separately in 2 and 3L-lidded plastic containers with some meat. With the emergence of third instar larvae, they were transferred to sawdust containers and then into 40x40x40 cages containing sugar or honey dissolved in water (5% solution), to transform into pupae and adults. To determine the shelf life of *L. sericata* eggs and larvae at refrigerator temperature, they were divided into sterile and non-sterile (without intervention) groups.

Results: Out of 5400 eggs, including 2700 sterile eggs and 2700 normal eggs, the highest percentage of eggs hatching referred to the sterile group (73%). However, there is no significant difference between the groups. The results of the study on the effects of sterility or non-sterility on the growth and development of the 1st instar larvae showed that no significant difference was observed between the two groups. The best time for eggs to hatch and their transformation into 1st instar was 72 hours and the best survival time of larvae was in 24 hours. The results obtained from this graph show that the most suitable temperature for eggs to hatch is 6°C and the most suitable temperature for survival of 1st instar larvae are 2 and 6 °C.

Conclusion: The results of this study showed, that the sterility or non-sterility of eggs and larvae cannot affect their durability.

Keywords: *Lucilia sericata*, Larval Therapy, Sterilization, Temperature, Time

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Introduction

Maggot debridement therapy (also known as larval therapy) is a type of biotherapy that involves the use of live, sterile maggots (flies' larvae) to clean up non-healing and soft-tissue wounds, treat the necrotic (dead) and sloughy tissue within the lesions (bio-debridement) and disinfection (1). This method represents a controlled myiasis in which necrophagous fly larvae will clean the wound and stimulate growth hormones, cell reproduction, and the body's immune system (2).

It is estimated that 1-2% of the population in developing countries will experience a chronic wound during their lifetime. History of medical treatments shows that sometimes attempting routine surgery or utilizing common antibiotics will lead to failure or don't show the predicted results in some cases. In such situations, Maggot Therapy, which has a history even older than many means utilized, would be considered (3). Wounds that do not respond to antibiotics or antibiotics cannot reach them due to the presence of rotten and infected tissue, are suitable options for maggot therapy. For the antibiotic to reach the wound, there must be blood flow and the infection must first be removed by surgery (4).

All fly larvae (maggots) are not suitable for larval therapy. Ordinary people previously used the accurate maggot species by relying on their experiences. Still, today, in such biodebridement treatment methods, many factors must be considered in selecting the correct species because of the critical matter of how these larvae will affect healthy and non-necrotic tissues (1). Sarcophagidae and some of Calliphoridae family such as *Chrysomya bezziana* can be mentioned among the species that should not be used because these species also swallow living tissue (5). The larva that is mostly used belongs to the Calliphoridae family especially the species *Lucilia sericata*, *Phormia regina*, and *Calliphora vicina*, which only feed on necrotic tissue. This group, commonly known as necrophagous flies has no harmful effects on healthy tissue (6).

For Maggot Therapy to be successful, maggots must be free of any bacteria before being placed on the wound. Another essential factor is the answer to the question of how and when we can keep and use these larvae to ensure that they will have maximum efficiency in treating wounds. Thus, this research was conducted to investigate the effect of being sterile or non-sterile on the hatching rate of eggs of *L. sericata*.

Methods

From September to October 2022, flies were collected in several sites in Tehran and Karaj County using a sweep net and bottle fly traps (reverse-cone model). Flies were identified by using valid systematic keys. The *L. sericata* eggs were placed separately in 2 and 3L-lidded plastic containers with some meat. With the emergence of third instar larvae, they were transferred to sawdust containers and then into 40x40x40 cages containing sugar or honey dissolved in water (5% solution), to transform into pupae and adults. The water container comprises a cotton wick and meat to feed adult flies. Then flies were transferred to special rearing cages, and fresh meat was placed for females laying eggs.

To determine the shelf life of *L. sericata* eggs and larvae at refrigerator temperature, 10,800 eggs, and larvae, including 5,400 eggs and 5,400 first instar larvae, were divided into sterile and non-sterile (without intervention) groups. The egg sterilization protocol was followed: firstly, separating the eggs from the laying location (inside the adult container), then washing eggs with normal water containing a little detergent. The eggs were washed with sterile distilled water, and sterilization with 1% sodium sulphite solution in physiological serum comprising 5.2% formaldehyde was done under the hood. The next step, which includes placing the eggs on the blood agar medium at predetermined temperatures and periods, was performed. The detachment of eggs from the blood agar medium and washing them using sterile distilled water were done, and, finally, they were kept in Eppendorf tubes or sterile plates until usage in the next stage of the experiment.

Results

Effect of being sterile or non-sterile on the hatching rate of eggs

Out of 5400 eggs, including 2700 sterile eggs and 2700 normal eggs, the highest percentage of eggs hatching referred to the sterile group (73%) (Table 1).

Table 1. Effect of sterile or non-sterile condition on the hatching rate of eggs of the *L. sericata*

Egg Condition	Egg Number	Hatching Percentage
Sterile	2700	73
Non-Sterile	2700	65.5
Total	5400	69.24

However, there is no significant difference between the groups (Figure 1).

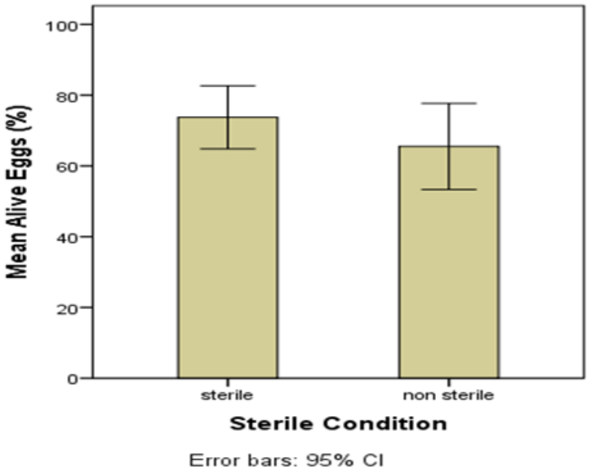

Figure 2. Effect of sterile or non-sterile condition on the hatching rate of eggs of the *L. sericata*

Effect of sterility or non-sterility on the growth and development of 1st instar larvae

The results of the study on the effects of sterility or non-sterility on the growth and development of the 1st instar larvae showed that out of 5400 1st instar larvae, including 2700 sterile and 2700 non-sterile, the highest percentage of development of the 1st instar larvae referred to the sterile group (77.56%) (Table 2).

Table 2. Effect of sterile or non-sterile condition on the rate of development of 1st instar larvae of the *L. sericata.*

1st Instar larvae condition	Number	Percentage
Sterile	2700	77.56
Non-sterile	2700	77.05
Total	5400	77.3

No significant difference was observed between the two groups (Figure 2).

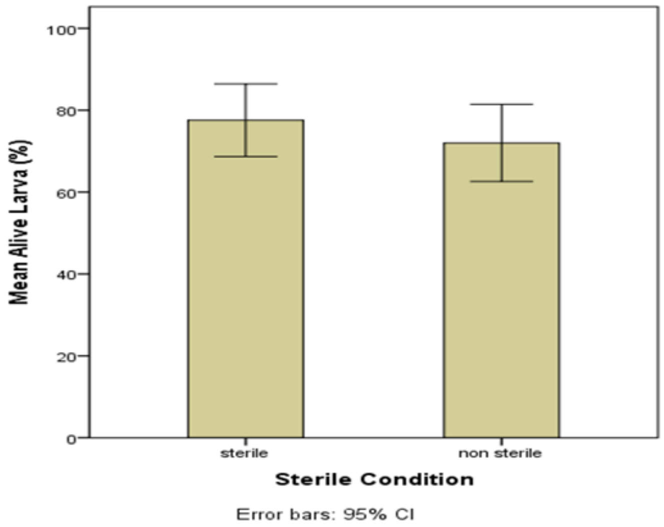

Figure 2. Effect of sterile or non-sterile condition on the rate of development of 1st instar larvae of the *L. sericata*.

Discussion

Calliphoridae family (blueflies) belongs to Diptera order and more than 1000 species of them are found all over the world. Some of the species in this family are of special importance from medical, veterinary, and legal. *L. sericata* have a high tendency to use necrotic tissues for their nutrition and do not attack living tissues. Therefore, they can be considered as suitable candidates for maggot therapy (7). Until now, much research has been conducted on the biology of *L. sericata* and it is considered the most important and most common species in maggot therapy research (8).

So far, many studies have been conducted regarding the use of sterile larvae in various chronic wounds. These larvae have proteolytic enzymes such as collagenase, trypsin-like, and chymotrypsin-like, which play a key role in breaking down the macromolecules on the surface of the wound (9). In addition, various important antimicrobial peptides have been identified from the secretory-excretory substances of these larvae, including Lucimycin, Lucifensin, and Lucifensin II, which seem to be effective in influencing the larvae during the treatment process (10). Also, recently, a series of heat-resistant hydrophilic compounds with a molecular weight of less than 3 kilodaltons have been identified from the hemolymph of *Calliphora vicina*, and these compounds produce antimicrobial peptides by affecting the fat body cells of the fly (11).

In this study, which was conducted to investigate the effect of sterile or non-sterile conditions on the hatching rate of eggs, 5400 eggs of *L. sericata* were evaluated in two groups of 2700 sterile and non-sterile eggs, and the results of the study showed that on average, 73% of sterile eggs and 65.5% of non-sterile eggs hatched and reached the stage of a larva, which is suitable for maggot therapy. Also, results showed that on average, 77.56% of sterile larvae and 77.05% of non-sterile larvae reached the second larval instar.

Conclusion

One of the most important stages of larval therapy is egg and larval sterilization, and this process mustn't affect the viability of the embryo and larvae. The results of this study showed, that the sterility or non-sterility of eggs and larvae cannot affect their durability.

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Ethics approval

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Conflict of interest

The authors report no conflict of interest in this study.

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