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Mass rearing of *Lucilia sericata* Meigen (Diptera: Calliphoridae)

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ABSTRACT

Objective: To carry out an experimental study with the main objective of mass rearing of sheep flies (*Lucilia sericata*). **Methods:** Hand collection and beef- or cattle liver-baited net traps were used for field fly sampling from April, 2010 to November, 2010. The samples collected from different places were placed in properly labeled tubes and sent to the Entomology Laboratory. Since maggot identification is important in inducing mortality, they were kept under insectary condition to develop to adult stage and identified using systematic keys. **Results:** A total of 218 flies were collected in three rounds of sampling from the field of Tehran and Karaj Counties. In the first generation, 433 flies including 135 (31.17%) male, and 298 (68.82%) female were yielded. The female/male of parent ratio was calculated as 1.72 in Tehran and in Karaj areas, whereas it was 2.20% and 1.81%, respectively in F1 and F2 generations, respectively. **Conclusions:** During this study, the mass rearing of sheep blow fly has been established at the School of Public Health, Tehran University of Medical Sciences and can be used for producing flies for maggot therapy.

1. Introduction

Wounds resulting from multiply factors, bed sores, diabetes, burns can be treated with *Lucilia sericata* (*L. sericata*) maggots[1]. But there are few data reported on green flies and diabetic patients in Iran[2], though diabetic foot ulcer is responsible for half of the diabetic patients visiting hospitals in Iran[3]. Following successful employment of fly larvae in treating antibiotic-resistant wound infections, the World Health Organization introduced maggot therapy as a suitable alternative method[4]. The larvae of *L. sericata* used in the treatment of wound would be potential treatment for infections and mass rearing program is the first step.

By now, 1 100 species of sheep flies were found in the world, while only 228 species were reported in the Neotropics, Africa and Southern Europe[5]. Temperate and tropical areas were reported as the main habitat of sheep blow flies. Layer of loose, litter, damp soil was reported the main habitat for maggot growing. Research centers should be actively involved in rearing larvae to provide the treatment centers.

Considering the potential existence of the necessary expertise and facilities in Tehran University of Medical Sciences to conduct larval therapy, construction and commissioning a specific section necessary for mass rearing of larvae was undertaken in order to maintain *L. sericata (Phaenicia sericata)* maggot under laboratory.

2. Materials and methods

2.1. Study area

This experimental study was carried out by sampling using net trap, hand collection and bait traps in appropriate places including gardens, livestock farms and

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slaughterhouses in Tehran and Karaj counties from April 2010 to November 2010.

2.2. Preparation of the first generation

The samples (larvae or adults) were caught from different ecological niches from plants and shrubs having fragrant flowers and sent to the Medical Entomology Laboratory at the School of Public Health, Tehran University of Medical Sciences in properly labeled containers encoded with data such as the date of collection, location, sampling method, temperature, humidity[6]. The beef and fish meat was used as bait for collection of flies in adult stage in open areas.

2.3. Identification of species

The identification of larvae was performed by examining the respiratory pores and posterior of cephalo-pharyngeal skeleton of the maggots which resulted in the larval death. Therefore, in this study larvae were kept to develop to adults and were anesthetized using CO_2, ether or cold shock and then identified[7-9].

2.4. Rearing

After identification, the fed adults were transferred into new cages for oviposition under the insectary condition of temperature (25±2) ℃, relative humidity of (45±5)%, and light / dark regime of 16:8 h[10,11]. Given that different populations from different regions prefer different physical conditions, they were reared under different temperature and humidity as well as different environmental conditions in order to achieve the optimum of temperature for the best yield. An electrical device was placed at the insectary to kill those flies trying to escape[12,13]. Layers of red meat were placed in the Petri dishes and tissue paper was placed between them, then a cap container with cut edge was placed on the meat[13]. Black pudding and horse blood agar were used as the simple food source for larval rearing[14-16]. Supervision of the rearing cages was occurred continuously and larvae were isolated from rearing age. After the emergence, the adults were placed in new cages and provided with essential food. The hatching dishes were placed to the larval medium and visited daily[17].

3. Results

Totally, 218 flies were collected in three rounds of sample collection (two times in Tehran and once in Karaj) and transferred to the School of Public Health. In the first and second sampling carried out in Tehran, 72.2% (52/72), 53.5% (46/86) of the specimens were female, respectively. In Karaj, 66.7% (40/60) of the samples were female and collected from slaughterhouse. The ratio of female parents (46) per eggs batch (9) was 5.1% in the second period of collection in Tehran strain. The number of eggs, larval, pupa and adults stages of F1 and F2 generation was shown in Table 1. A total of 433 flies were yielded in the first generation including 135 (31.17%) male, and 298 (68.82%) female. From a total of 298 adult female flies transferred to cages, 13 batch eggs were obtained, and grown to larvae, pupae, and adult stage. In F1 generation, mortality rates of larvae were calculated as 2.72%, 1.72%, and 7.63% in the 1st, 2nd, and 3rd instars, respectively. Mortality rate was found 3.35% during the pupae stage of F1 generation. Mortality rates of larvae in F2 generation were 3.47%, 6.28%, and 7.56% in the 1st, 2nd, and 3rd instars, respectively. The female/male ratio was 2.20 and 1.81 for F1, and F2 generation, respectively.

Table 1
Number of eggs, larval stage I, II, III, pupa and adults of *L. sericata* in F1 and F2 generation in Tehran Insectary, 2010 [*n* (%)].

Generation	Egg batch	Larval stage			Pupa	Adult		
		I	II	III		Male	Female	Total
F1	9	479 (97.28)	466 (98.30)	458 (92.37)	448 (96.65)	135 (31.17)	298 (68.32)	433
F2	13	462 (96.53)	446 (93.70)	437 (92.44)	404 (91.33)	131 (35.50)	238 (64.45)	369

4. Discussion

The period before oviposition is highly affected by temperature and humidity of the nurturing medium, therefore all pregnant female flies should be kept under optimum conditions of temperature and humidity. The optimal temperature for larval development is (35-38) ℃, though larval survival is the greatest at (17-32) ℃. Larvae complete their development in 4 to 13 days at optimal temperatures, but require 14 to 30 days under temperatures of (12-17) ℃[10,11]. The first generation of the wild flies laid egg batches later because the parents were not readily accommodated with the new conditions. Latency in mating in the first generation may be a contributing factor to this situation. In the same study, the period of cycle from egg to adults was reported to be 1-2 weeks, whereas in our study, it was calculated as 2-3 weeks[13,18]. Agree with this study, in the second generation,

the period from egg to adult was reported longer than the first generation[13]. Changes in diet and nutrition may be contributing factors to this finding. In our study, during the F2 generation, less egg hatched successfully. Agree with our study, Sherman in 1996, and Wolf in 2005 stated that less larvae were yielded in the second generation maybe due to photoperiod, temperature, humidity, food and nutrition[13,18]. The impact of food type on the development of larvae of *L. sericata* suggests that there are significant differences between feeding of larvae on beef, liver and humburger. The size of larvae fed on hamburger was smaller than those fed on beef and sheep liver. In agreement with the results of our research, higher number of *Lucilia* larvae was yielded when adults were fed on beef compared with chicken flesh and chicken liver[19]. The results of this experiment is in agreement with the results of Day and Wallman in 2006. They stated that the growth rate of larvae fed on sheep liver was significantly lower than those grown on the chicken and beef flesh[19]. According to these studies, food type is an essential factor on growth and development of green flies. In our study, the duration of adult stage, mating and spawning was found 21 days whereas it was reported two weeks in other studies[13,18]. The longevity of adults in the second generation under insectary condition indicated that the mortality of gravid females was higher than non gravid and males. In our research, it was found that the 1st and 2nd instars were quite sensitive to dry and food shortages whereas the 3rd instars larvae were sensitive to humidity.

In conclusion, mass rearing of *L. sericata* larvae can be used to prepare the appropriate treatment for diabetic ulcers, burns and wounds that do not respond to antibiotics. On the other hand, this method can be used in other parts of the world under the supervision and approval of health authorities.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Behzadi M, Yazdanpanah Poor H. The use of fly larvae to treatment of some myasis. The 15th Iranian Veterinary Congress. 26-28 April 2008, Tehran, Iran.

[2] Salimi M, Goodarzi D, Karimfar MH, Edalat H. Human urogenital myiasis caused by *Lucilia sericata* (Diptera: Calliphoridae) and *Wohlfahrtia magnifica* (Diptera: Sarcophagidae) in Markazi Province of Iran. *Iranian J Arthropod Borne Dis* 2010; **4**(1): 72-76.

[3] Jan Nsary Ladani M, Mirab-Zadeh A, Mashayekhi M, Sharifi SH. *The final report of the project of using of larvae Lucilia sericata to treatment of infectious wounds.* 1st ed. Tehran: Scientific and Industrial Research Press; 2006, p. 1-51.

[4] Bani-Ardalans M. The rearing of maggot in the insectarium. *Weekly Med Rep* 2005; **578**: 1-15.

[5] Hall MJ. Trapping the flies that cause myiasis: their respones to hoststimuli. *Ann Trop Med Parasitol* 1995; **89**: 333-357.

[6] James MT. *The flies that cause myiasis in man*. New York: Chapman & Hall; 1947, p. 1-428.

[7] Zumpt F. Calliphorinae. In: Lindner E. (ed.) *Die fliegen der palaearktischen region*. Berlin: Germany Department of Agriculture; 1959, p. 1-140.

[8] Crosskey RW, Lane RP. *House- flies, blow-flies and their allies (Calyptrate Diptera)*. London: Chapman& Hall; 1993, p. 1-428.

[9] Barnard DR, Geden CJ. Influence of larval density and temperature in poultry manure on development of the house fly (Diptera: Muscidae). *Environ Entomol* 1993; **22**: 971-977.

[10] Lysyk TJ. Effects of temperature, food, and sucrose feeding on longevity of the house fly (Diptera: Muscidae). *Environ Entomol* 1991; **20**: 1176-1180.

[11] Greenberg B. *Flies through history*. New York: Princeton University Press; 1973, p. 1-447.

[12] Sherman R, Wyle F. Low-cost, low-maintenance rearing of maggot in hospitals, clinics and school. *Am J Trop Med Hyg* 1996; **54**: 38-41.

[13] Spiller D. Insect colonization and mass production. In: Smith CN. (ed.) *House flies*. New York: Academic Pres; 1966, p. 1-31.

[14] Sherman RA, My-Tien Tran JM. A simple, sterile food source for rearing the larvae of *Lucilia sericata* (Diptera: Calliphoridae). *Med Vet Entomol* 1995; **9**: 393-398.

[15] Cetinkaya M, Ozkan H, Koksal N, Coskun SZ, Girigin HM. Neonatal myiasis: a case report. *Turk J Pediatr* 2008; **50**: 581-584.

[16] Fleischmann W, Grassberger M , Sherman R. *Maggot therapy: a handbook of maggot-assisted wound healing complementary medicine*. 1st ed. Berlin: George thieme Verlag Germany; 2004, p. 1-93.

[17] Wolff H, Hansson J. Rearing larvae of *Lucilia sericata* for chronic ulcer treat meant - an improved method. *Acta Derm Venereol* 2005; **85**: 126-131.

[18] Hassan A. Influence of food type on larval growth in *Lucilia sericata*. London: School of Biosciences, University of Westminster; 2008, p. 1-26.

[19] Wallman JF, Day DM. Influence of substrate tissue type on larval growth in *Calliphora augur* and *Lucilia cuprina* (Diptera: Calliphoridae). *J Forensic Sci* 2006; **51**: 657-663.