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Article

Formulation and evaluation of the efficacy of an artificial diet for two forensically-important flies

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Abstract: The forensically-important Dipteran flies, *Lucilia sericata* and *Megaselia scalaris*, complete their life-cycles in perishable, filthy, unhygienic and fowl-smelled natural diets, which hinder the rearing work and the indoor environment of laboratory. To overcome this unhygienic condition, a newly formulated simple, comparatively more hygienic one was prepared and evaluated for the rearing of these two flies. This artificial diet, when compared to their natural diets for rearing at different temperatures, it demonstrated no significant difference for their developmental durations. Statistical analyses proved that the difference between the natural and formulated diet was not responsible for their developmental durations; rather their rearing temperatures played a significant role in this respect. Taken together, these findings showed that the newly formulated artificial diet might facilitate the hygienic and easy rearing of these flies for forensic and medical entomology as well as other research purposes in terms of nutrition, cost-effectiveness and availability of the ingredients of diets.

Keywords: artificial diet; *Lucilia sericata*; *Megaselia scalaris*; temperature; developmental durations

1. Introduction

Forensic entomology is the medico-legal area of entomology, which applies insects and other arthropods in criminal investigations (Catts and Goff, 1992). Usually insects colonize in a decomposing vertebrate corpse or carrion (Amendt, 2004) and these colonizers are used to estimate the time of death i.e., time elapsed since death, also known as post mortem interval (PMI). Forensic entomology also reveals the movement of the corpse, manner and underlying causes of death as well as the association of suspects at the death scene (Joseph *et al*., 2011) through the study of the life cycles of insects particularly of the order of Diptera and Coleoptera.

The Dipteran fly, *Lucilia sericata*, which belongs to the family of Calliphoridae, plays an important role in forensic, medical and veterinary sciences. The immature flies are used to estimate the minimum portion of the PMI, in a multitude of settings (Rueda *et al*., 2010). *L. sericata* is one of the first insects to arrive at a corpse and according to Byrd and Castner (2009), Calliphorids appear on carcasses in experiments within minutes of death and are reported as the most important species in providing accurate PMI estimations.

Another fly, *Megaselia scalaris* belongs to the family of Phoridae and has also been applied in forensic medicine to estimate PMI (Disney, 2008; Noor, 2010). *M. scalaris*, which *is* also called "Scuttle Fly", is an excellent organism to study genetic mechanisms. The fly may be used to demonstrate the general principles of sex determination (Traut, 1994), and molecular and developmental genetics admirably (Dama, 2014).

However, suitable larval diet and optimum environmental status are very essential for successful rearing of these flies in laboratory conditions. Natural diets are usually readily perishable, filthy, unhygienic and generate foul smell leading to the restriction of rearing in laboratory (Sherman and My-Tien Tran, 1995). On the other hand, artificial diets are comparatively hygienic, devoid of foul smell; possess consistent quality and a good cost

benefit relationship. It is easy to collect ingredients for artificial diets from commercial sources all the year round, and they are generally more economical than natural food materials (Rock *et al*., 1967).

Although a voluminous number of investigations on the forensically-important carrion associated arthropods have been reported throughout the world, in Bangladesh such reports are very few in numbers. Due to lack of standardization of laboratory diets, feeding, breeding and rearing protocols of insects, research on behavioral, medical and forensic entomology has become problematic. Therefore, to resolve the above-mentioned crises a new artificial diet has been formulated, and its efficacy and availability has been evaluated in this study to replace the fowl-smelled natural diets commonly used for rearing of these two forensically-important flies in the laboratory conditions.

2. Materials and Methods

2.1. Location of the experiment

This study was carried out at the Laboratory of Medical and Forensic Entomology at the Department of Zoology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

2.2. Collection and rearing of flies

The adults of blow fly, *L. sericata* and the scuttle fly, M. scalaris were collected from the university campus by using the chicken and fish leftovers. Since Bangladesh experiences an average approximate temperature (27 ± 1°C in summer) and (15 ± 1°C in winter), both the flies were kept in incubator in cages covered with mosquito nets and were reared at 27 ± 1°C and 15 ± 1°C separately on natural and artificial food. Same artificial diets were formulated for both of the flies and were reared at different temperatures along with their natural diets to understand the efficacy of the newly formulated artificial diets (see the section 2.3 and 2.4).

2.3. Sources of natural diet

The leftovers such as liver, guts, and skins of chicken or whole fish were supplied to the flies as their natural diets. The diets were taken into a pored container to allow the gaseous exchange. From the oviposition to the emergence as adult, the experiment was observed several times in a day. The life cycle was studied from the readings of the observation. Care was taken to overcome the blockage of gaseous exchange. A scanty amount of water was applied to the diet time to time to avoid desiccation and a piece of cloth was tied on top of the container so that the larvae could not escape.

2.4. Formulation of artificial diet

The ingredients of artificial diet were chicken egg, nutrient agar, and whole powdered milk. The ratio of raw egg with yolk, milk, and nutrient agar was formulated in gram (g) as (85.0): (7.5): (7.5). This combination was stirred with a glass rod properly in a plastic cup. When the diet was taken into a plastic cup and exposed to the adults for oviposition in it. On appearance of the larvae, the cup was covered with a piece of cloth to prevent their escaping from the cups. A scanty amount of water was applied to the diet time to time to avoid the desiccation of the food. From the oviposition to the emergence as adult, the experiment was also observed several times in a day. Temperature data were recorded every time during the observations.

2.5. Statistical analysis

The t-test were conducted to test the significance of developmental durations of both flies reared on natural and artificial diets in laboratory conditions. Additional t-test were carried out to examine the significance of developmental durations at different condition of temperatures on natural and artificial diets in laboratory conditions. All of the analyses were conducted using Microsoft Office Excel 2007.

3. Results and Discussion

Both of the flies usually feed on human foodstuffs, decayed, fermenting or rotting organic material of either animal or vegetable origin, which include garbage and waste from food processing and thus transport various disease agents. These fly species have adapted to human settlements and have been associated with the transmission of enteric infections (Rozendaal, 1997). These altogether release foul smell leaving totally unhygienic as well as uncomfortable atmosphere in laboratory conditions and pose a big challenge for their rearing for research purposes. Moreover, it has been reported that the toxins produced from the decomposing tissues of natural diets can affect the development rate leading to the errors in PMI estimates for forensic studies in the laboratory (Estrada *et al*., 2009). These might be fundamental drawbacks for conducting the research work smoothly. To overcome this challenge a nutritious and hygienic diet is very urgent which is equivalent to

their natural diets. Therefore, in the present study a new artificial diet was formulated for both *L. sericata* and *M. scalaris* in laboratory conditions, and the artificial diet was compared to natural diet for the duration of developmental time for rearing of the flies.

One study carried out by Clark *et al*. (2006) illustrated the comparison of development of blowfly, *L. sericata* between fed on lung, liver and heart from both cows and pigs. From the observation, it was reported that during rearing on lung and heart compared to liver, larva grew much faster and gave rise to comparatively larger adults. Similarly, it was also found that these flies completed their life cycle significantly more rapidly and got larger when reared on pig compared to cow tissue. Since natural diets might provide varying nutrition on the basis of different animal source, the proposed artificial diet by this study might be functional to avoid the alteration regarding the duration of life cycle of forensically important flies.

To compare the efficacy of the artificial diet, we considered the role of temperature because according to the previous research temperature is one of the important factors (Shiravi *et al*., 2011). The *developmental* durations of both of these forensically important flies, *L. sericata* and *M. scalaris* were observed at different temperatures on both natural and on newly formulated artificial diet in the laboratory conditions (Figures 1 and 2) to evaluate the efficacy of the artificial diet. Increase of temperature (up to a certain level) was found to shorten the duration of the development of these flies while decreasing of temperature delayed the duration (Figures 3 and 4).

Table 1. Comparative compositions of natural and artificial diets (100 g) for the flies.

Name of fly		Natural diet	Ratio of ingredients of artifial diet (g)		
English name	Latin name		Egg	Milk powder	Agar
Blow fly	*Lucilia sericata*	Chicken leftover, fish	85.0	7.5	7.5
Scuttle fly	*Megaselia scalaris*	Chicken leftover, fish	85.0	7.5	7.5

Table 2. Comparative developmental durations of both flies at 15 ± 1°C and 27 ± 1°C on natural and artificial diets.

Name of fly		Type of diet	15 ± 1ºC			27 ± 1ºC		
English name	Latin name		Developmental durations#	Mean (days)	Level of significance	Developmental durations#	Mean (days)	Level of significance
Blow fly	*Lucilia sericata*	Natural	13, 16, 17	15.3	$p = 0.73$	8, 9, 10	9	$p = 0.41$
		Artificial	14, 16, 18	16.0	$p^* > 0.05$	9, 10, 12	10.3	$p^* > 0.05$
Scuttle fly	*Megaselia scalaris*	Natural	15, 17, 18	16.6	$p = 0.41$	8, 9, 11	9.3	$p = 0.41$
		Artificial	16, 18, 20	18.0	$p^* > 0.05$	8, 11, 12	10.3	$p^* > 0.05$
# indicated three replications and * indicated statistically not significant								

Figure 1. Pupae of *L. sericata* and *M. scalaris* on natural diet.

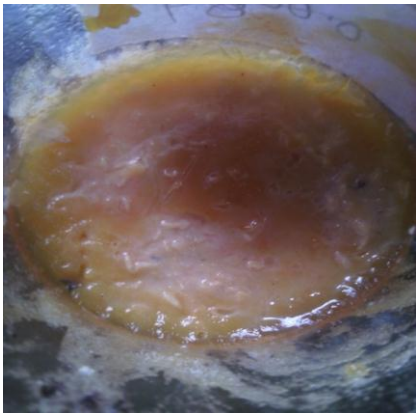

Figure 2. Larvae of *L. sericata* and *M. scalaris* on artificial diets.

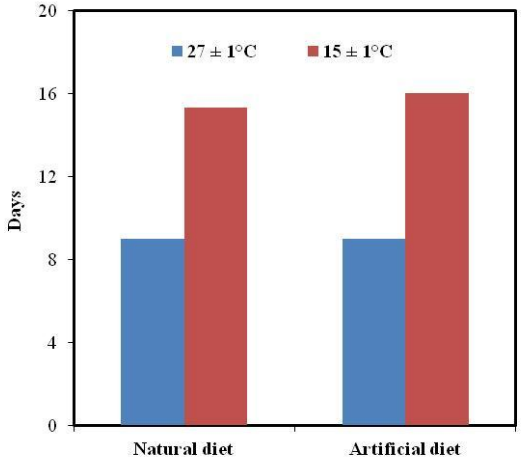

Figure 3. Role of temperature and diet on the developmental durations of *L. sericata*.

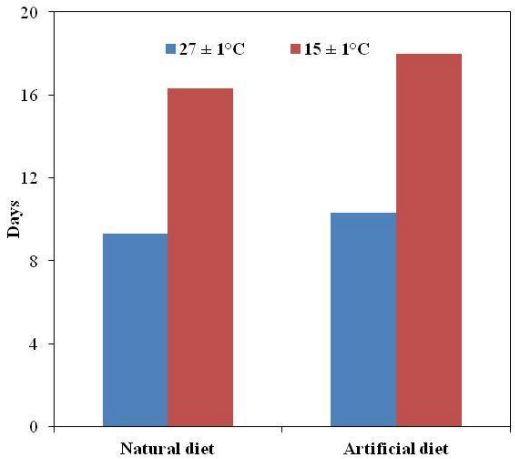

Figure 4. Role of temperature and diet on the developmental durations of *M. scalaris*.

The development of blow fly at 27 ± 1°C on natural and artificial diets in laboratory condition demonstrated similar performances. Although it was recorded that developmental times were slightly shorter in case of natural diet, statistical analysis revealed no significance ($p > 0.05$) (Table 2). Likewise, the difference of developmental times of this fly at 15 ± 1°C on natural and artificial diets also did not show statistical significance ($p > 0.05$) (Table 2).

In case of scuttle fly, when the developmental times were studied at 27 ± 1°C on natural and artificial diets in laboratory conditions, the difference was insignificant for scuttle fly as well ($p > 0.05$) (Table 2). However,

when the developmental times were studied at 15 ± 1°C, *p*-value was > 0.05 which was the same as at 27 ± 1°C (Table 2). Thus, it can be said that there was no significant difference for developmental durations of both of the flies when reared on between natural and artificial diets but revealed at different temperatures.

On the other hand, when the developmental times of blow fly were studied on natural diet at 27 ± 1°C and 15 ± 1°C in laboratory conditions, it was observed that temperature played a significant role on the duration of their developmental times. This study showed that the developmental times of blow fly were shorter at 27 ± 1°C compared to that at 15 ± 1°C showing the significant difference ($p = 0.01$) (Table 4). In the same way, when the developmental times were studied on artificial diet, temperature also played the significant role here as well ($p = 0.03$) as shown in the Table 2 and Figures 3 and 4.

Turning to the scuttle fly, when the developmental times were studied on artificial diet at 27 ± 1°C and 15 ± 1°C in laboratory conditions, it was observed that temperature played a significant role on duration of their developmental times as developmental times were shorter at 27 ± 1°C compared to that at 15 ± 1°C showing the significance of the difference ($p = 0.04$) (Table 5). Similarly, when the developmental times were studied on artificial diet, temperature played the significant role which is the same as on natural diet ($p = 0.047$) (Table 2; and Figures 3 and 4).

Thus, it might be assumed that, rather than natural or artificial diet, temperature played the critical role to the duration of the developmental times for both blow and scuttle flies. In this study, depending on the types of diet, the life cycle spanned from egg to eclosion in blowfly was 8-12 days, whereas it was 8-17 days in the scuttle fly at 27 ± 1°C and 15 ± 1°C, respectively. In different studies, the effect of diet has been found significant on development of flies (Kaneshrajah and Turner, 2004; Tarone, 2006; Clark *et al*., 2006; Reibe *et al*., 2010). In the current study, in case of *L. sericata*, on an average, total 8-12 days were needed to complete the life cycle which was the same as information provided by Shiravi *et al*. (2011) (8-12 days) and to some extent the duration of life cycle was harmonious also to the data of Kamal (1958) (12-15 days at 22°C and 50% relative humidity). However, this duration is quite shorter compared to data provided by Rueda *et al*. (2010) (14 days), Anderson (2000) (14 days at 27° C), Nuorteva (1977) (23-28 days under field conditions), Usaquén and Camacho (2004) (26 days under natural environmental conditions), and Anderson (2000) (32 days at 16°C and 20 days at 21°C). These discrepancies reveal that to complete a life cycle faster, our artificial diet is no longer unlikely compared to natural diet.

Shin-Ichiro and Numata (2001) reared the larva of blow fly, *L. sericata* (Diptera: Calliphoridae) on artificial diet and found insignificant difference between larva reared on that artificial diet and those reared on beef liver. However, their artificial diet was much more complex consisting of almost 7 elements compared to our artificial diet which is easier to prepare and more simple made of only chicken egg, milk powder and agar. The durations of developmental time due to forensic flies vary regarding the populations of different geographical regions (Rechard *et al*., 2008; Gallagher *et al*., 2010). As our study was conducted based on forensic flies of our own country, these data would be found extremely important for Bangladeshi entomologists during governing researches involving forensic entomology.

The duration of the developmental time for *M. scalaris* described in this research is 9-11 days at 27 ± 1°C temperature which is more or less similar compared to data provided by Dama (2014) (10 days at 22-24°C). The other study under taken by Disney (1994) on scuttle fly showed the differences of developmental time even in every stage of life cycle regarding temperature. The findings were likely that the 1st instar, 2nd instar and 3rd instar took 1-2, 1-2 and 3-4 days consecutively and a more 1-2 days were needed before pupation when the flies were exposed at 22-24°C.

Different diets have different effects on *M. scalaris* regarding developmental time and size as well as adult emergence and longevity (Idris *et al*., 2001; Zuha *et al*., 2012). This is one of the reasons, we prepared a standard diet on which *M. scalaris* can easily be reared for research and many more works related to forensic entomology. Shefa *et al*. (2013) also designed an artificial diet comprised of whole milk powder, bovine blood, chicken eggs and wheat bran and tried it on the growth and development of a forensic fly, *L. cuprina*. They also didn't find any significant difference on the developmental durations during studies. However, they assessed their studies at 25°C only but our study was conducted at different temperatures.

These results clearly demonstrated that temperature exerted a significant effect on the duration of the developmental times of both the forensically important flies, while the diets showed insignificant role. The artificial diet formulated in the experiment was sufficiently nutritious for the development of both of these flies in laboratory conditions compared to the natural diets. However, proper handling and hygiene must be maintained strictly while rearing them in the natural diet. Since the preparation of the artificial diets and the rearing protocol is simple, hygienic, less perishable and smell-free, it can be easily incorporated into forensic and medical entomology as well as other relevant research programs.

4. Conclusions

Since the natural diets of the flies generate foul smell and unhygienic conditions in the laboratory, we formulated and evaluated a comprehensively more hygienic, cost-effective and nutritious diets for the rearing of two forensically-important flies. Further investigations are required to establish this formulation as the most effective dietary sources of these important flies for scientific investigations.

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Conflict of interest

None to declare.

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