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Effect of Temperature on the Breeding Black Soldier Fly Larvae in Vitro for Basic Health-oriented Research

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Background & Aims of the Study: The prevalence of food insecurity in many countries and the challenges emerging to feed more than 9 billion people by 2050 have led the researchers to look for alternative sources of protein in human and animal diets. In this regard, today, the use of insects has attracted a lot of attention since they contain high nutritional value and help to preserve environmental resources. Among the various species of insects, particular attention has been paid to the black soldier fly (BSF) since it can consume from a variety of substrates, including organic waste. Various factors, such as temperature, humidity, density, light, and diet, are involved in the breeding of this insect. It seems that temperature is more effective in the breeding stages of this species than the other factors. Due to the insufficient information on finding the optimal temperature in breeding this species, this study was conducted to determine the mentioned factor in the maximum production and reproduction of black soldier flies to eliminate organic waste and turn it into valuable material in animal food.
Materials and Methods: Organic waste, including kitchen fruit and food, was used to feed the larvae. Adult flies were then reared in cotton net cages (40×40×40 cm) and under the temperature range of 25-35°C. Afterward, the eggs were collected by fine needles and transferred to a temperature-controlled incubator during the hatching stage to undergo experiments in the specified temperature range (i.e., 25-35°C). The larvae fed freely from the formulated diet (i.e., chicken feed) until the pre-pupal stage. The produced pupae were monitored for growth and survival in the temperature range of 25-35°C. The emergence of adult BSFs at different temperatures was examined after the completion of the pupal stage under controlled temperature. In this descriptive study after the completion of each insect's development stage, the percentage of insect survival in each stage of measured temperature condition was determined by estimating the proportion of the attribute present in the population.
Results: According to the results of this study, the highest hatching percentage (80%) was recorded at 30°C for 4 days, while the slowest growth period was obtained at 30°C for 13 days with a survival rate of 92%. It was also revealed that the highest pre-pupal and pupal survival rates were 82% at 30°C for 10 days and 77% at 30°C for 7 days, respectively. The lifespan of adult flies at 30°C was reported to be 9 days. The statistical population of this consisted of 300 pupae at each temperature. The survival percentage was reported after the survived pupae were counted.
Conclusion: The results of this study showed that the growth and reproduction of BSFs were significantly affected by temperature. In this study, the optimum temperature in the breeding of BSFs was obtained as 30°C. Temperature can also affect the insect's biological life cycle, such as immaturity survival and adult lifespan, growth, fertility, gender ratio, and population growth parameters.

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Background

The prevalence of food insecurity in many countries and the challenges emerging in the future to feed more than 9 billion people by 2050 have led researchers to look for alternative sources of protein in human and animal nutrition (1). The increasing population in the world will lead to the growing demand for animal source foods in the future (2). It has been anticipated that the request for meat consumption will be increased by 76% by 2050 worldwide (3), which according to prospective studies, this increase seems to be higher in developing countries (113%) than in developed countries (27%) (4).

More than one-third of the world produced grain is being fed to livestock annually (5), which can feed at least three billion people worldwide (5). Based on the statistics, approximately a third of the food produced for human consumption is wasted, rendering for a total of 1.3 billion tons of food per year. Food loss is indirectly associated with a wide range of environmental effects, such as soil erosion, deforestation, water and soil pollution, and greenhouse gas emission (6).

Today, the use of insects as a rich source of protein has attracted much attention as an animal feed alternative. The use of insects can be considered a good option as a sustainable feed in the aquaculture, animal husbandry, and poultry industry since they contain high nutritional value and help to preserve environmental resources (2, 7-9). Among the various species of insects, particular attention has been paid to the black soldier fly (BSF) since it can consume from a variety of substrates, including organic waste (10-12). The ability of this insect to convert organic waste into high-quality nutrients has opened an innovative economic prospect in breeding this insect. The protein produced by insects is a proper alternative to fish powder or soybean meal in providing food for livestock (13), pets, such as dogs and cats (14), poultry (15), and fish (16). *Hermetia illucens*, the BSF, is a saprophytic fly native to the Neotropics region. The distribution range of these flies has changed over time and today covers the warmer regions of the world (17). According to the Food and Agriculture Organization, insects can reduce world hunger and improve global health by reducing greenhouse gas emissions and air pollution levels. Therefore, great demands have been made for breeding this insect to manage organic waste (18). The larvae of the black soldier fly prevent the reproduction of pests and houseflies (19) In addition, unlike houseflies, they do not attack human home environments and are not carriers of diseases and pests on farms (20, 21).

Special biochemical properties in the digestive enzymes of this insect facilitate the digestion of organic waste (22), among which the enzyme trypsin plays an important role (22). Furthermore, the intestinal microbiome in larvae has a significant part in the conversion of organic waste (23, 24). The larvae of this insect are classified as saprophytic insects that feed on organic industrial, agricultural, and household waste or dead plant and animal waste (25) Black soldier fly larvae contain considerable amounts of protein (63-37%), fat (20-40%), calcium, iron, potassium, magnesium, phosphorus and zinc, niacin, vitamin B12, thiamine, and riboflavin (26, 27).

These larvae can be fed and reared in the bed of organic waste, including food waste (28), spent mushroom compost (29), household organic waste (30), chicken manure (31), animal manure (32), and sewage sludge (33). The results of some studies revealed that BSF larvae can reduce waste produced by pigs, chickens, and kitchens by 51-80% (34). Today, a great interest has been developed regarding the new methods of mass production of this insect due to its various benefits in the management of organic waste, as well as being a suitable source of sustainable protein in animal feed. However, the lack of scientific data and the reluctance of commercial producers to share accurate information have made smallholder farmers face disruptions in the large-scale BSF production technology (35).

Different factors, such as temperature, humidity, density, light, and diet are involved in the breeding process of this insect (36). Various studies have been conducted to broaden the knowledge of breeding black soldier flies. Accordingly, the temperature was found to be the most effective factor on the development stages and life of this species, compared to the other ones (37, 38), directly influencing the quality and quantity of insects produced in breeding centers (39). Nevertheless, there is insufficient information on finding the optimal temperature in BSF breeding to maximize its mass production. Considering these reasons, this study was performed to determine the optimal temperature for maximum BSF production and reproduction to eliminate organic waste and convert it into a valuable source of animal feed.

Materials & Methods

In order to feed the larvae, organic waste, including fruit and kitchen waste, was prepared, grounded, and dried up to the removal of 50% of the excess water. All stages were tested at 50% humidity.

Black soldier fly breeding

Several cotton net cages (40×40×40 cm) were prepared to breed adult flies. Since flies naturally feed on flower nectar, a container holding pieces of cotton soaked in water and sugar were used in this study for feeding them. The breeding was carried out at 28°C, 70% humidity, 6,500 M^3 density in the cage, light duration of 6 h with the light intensity of 60 µmol/s/m, and light-emitting diodes were used to stimulate mating The flies were fed for 7 days they reached puberty and their body changed completely to black. Black soldier fly eggs were laid in the laying medium, which was made of fluted corrugated cardboard or chopped wood. The spawning site was placed on the organic waste bed next to which flies laid eggs for the survival of the offspring.

Freshly laid egg clusters were daily collected from the colony. Adult BSF pairs were placed in cotton cages, in each of which flies fed on sugar water on soaked cotton balls. The relative humidity was set at 50%. Subsequently, the flies gathered near the bed and laid eggs .The cluster(s) of daily produced eggs were examined under a microscope, while the lifespan of flies was also recorded. Adult's lifespan was calculated based on how long a fly lived from the adult stage to death.

Eggs hatching

The eggs were randomly collected with fine-tipped needles and transferred to petri dishes (150 mm). Afterward, petri dishes containing eggs were moved to the incubator. The eggs were examined daily at regular intervals of 6 h until hatching. The incubator under controlled thermal conditions (25-35°C) was used to perform the determined experiments in each of the temperature conditions. In each incubator, the effect of temperature on the growth pace and survival of eggs was investigated.

Larval breeding

In each temperature treatment, the larvae were randomly placed on a formulated diet (chicken feed) in rectangular plastic containers. The opening on the lid of the containers was covered with a net to permit adequate ventilation. The larvae then fed freely until the pre-pupal stage.

Pre-pupal and pupal breeding

In this stage of the study, 1-hour newly formed pre-pupae (n=300) were randomly selected in each temperature treatment with three replications. Subsequently, they were placed separately in small plastic containers, as pupal beds, covered with wet sawdust (2.5 cm deep). These containers were daily monitored for growth and survival at the pupal stage. Afterward, all 300 pupae were transferred separately to plastic containers covered with a layer of wet sterile sawdust (2.5 cm deep) for emergence. The adult emergence periods were examined at different temperatures. In this descriptive study after the completion of each insect's development stage, the percentage of insect survival in each stage of measured temperature condition was determined by estimating the proportion of the attribute present in the population.

Results

Egg

Based on the results of this study, temperature showed different effects on the growth and survival at immature and adult life stages. Moreover, it was revealed that the duration of hatching (birth) was significantly different at different temperatures. Accordingly, the duration of hatching eggs was found to be 9 days at 20°C, 6 days at 25°C, 4 days at 30°C, and 2.5 days at 35°C (Figure 1). The hatching percentage of eggs at 20°C was 50%, which was very low compared to the other temperatures. The highest percentage of hatching (80%) was recorded at 30°C (Table 1).

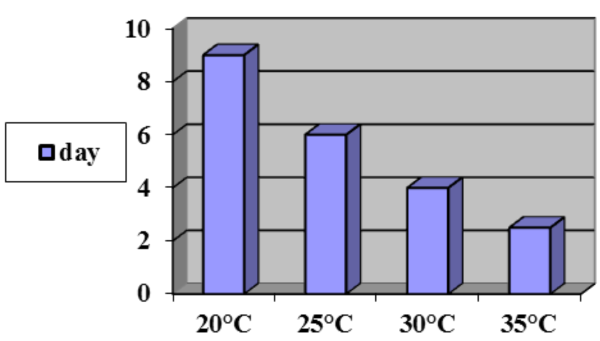

Figure 1) Duration (day) and percentage of larval hatching

Table 1) Duration (day) and percentage of larval hatching

Temperature	20°C	25°C	30°C	35°C
Hatching duration	9	6	4	2.5
Hatching percentage	50%	55%	50%	70%

Larva

The results showed that larval growth period was different at the studied temperature range; meaning that it was calculated at 30 days at 20°C, 18 days at 25°C, 13 days at 30°C, and 16 days at 35°C. The longest and the shortest larvae growth periods were recorded at 20°C (30 days) and 30°C (13 days), respectively (Figure 2). Larvae survival percentage was significantly different in various temperature treatments, showing the lowest at 20°C with a 70% survival rate. It was also that this rate was 93% at 25°C, 92% at 30°C, and 90% at 35°C (Table 2).

Pre-pupal

The pre-pupal growth period was signify-cantly different at the studied temperature range.

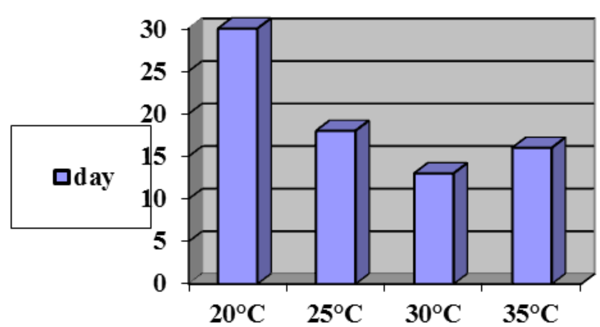

Figure 2) Duration (day) of larval growth and its survival percentage

Table 2) Duration (day) of larval growth and its survival percentage

Temperature	20°C	25°C	30°C	35°C
Growth duration	30	18	13	16
Survival percentage	70%	93%	92%	90%

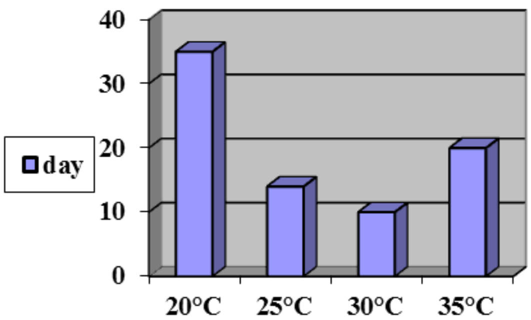

Figure 3) Duration (day) of pre-pupal growth and its survival percentage

In this respect, pre-pupal growth duration was obtained as 35 days at 20°C, 14 days at 25°C, 10 days at 30°C, and 20 days at 35°C. The fastest and lowest pre-pupal growth paces were recorded at 20°C (35 days) and 30°C (10 days), respectively (Figure 3). According to the findings, the highest pre-pupal survival rate was found at 25°C and 30°C with 83% and 82% rates, respectively. On the other hand, the lowest survival rate was estimated at 20°C and 35°C with rates of 68% and 75%, respectively (Table 3).

Pupa

Based on the results, there was a significant difference in pupal growth duration in the studied temperature range. In this regard, the pupal growth period was found to be 15 days at 20°C, 9 days at 25°C, 7 days at 30°C, and 6 days at 35°C. It was also revealed that the fastest pre-pupal growth pace was recorded at 20°C (15 days), while the slowest growth pace was at 35°C (6 days) (Figure 4). The highest and lowest pupal survival rates were obtained at 30°C and 20°C with 77% and 61% rates, respectively. This rate was 65% and 67% at

Table 3) Duration (day) of pre-pupal growth and its survival percentage

Temperature	20°C	25°C	30°C	35°C
Growth duration	35	14	10	20
Survival percentage	68%	83%	82%	75%

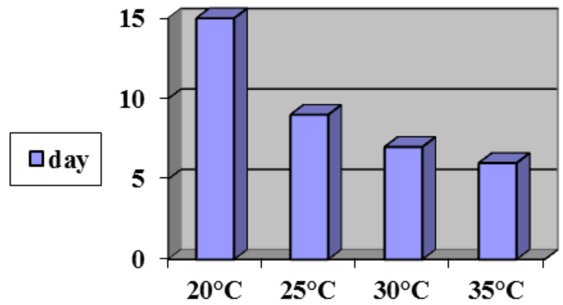

Figure 4) Duration (day) of pupal growth and its survival percentage

Table 4) Duration (day) of pupal growth and its survival percentage

Temperature	20°C	25°C	30°C	35°C
Growth duration	15	9	7	6
Survival percentage	51%	67%	77%	65%

35°C and 25°C, respectively (Table 4). It was also revealed that the total growth period of larvae up to the adult stage varied from 34 days at 30°C to 89 days at 20°C.

Adult

The lifespan of adult BSF was significantly affected by temperature, which was found to be 14 days at 25°C, 9 days at 30°C, and 4 days at 35°C. The longest and shortest lifespans were recorded at 25°C (14 days) and35°C (4 days) (Figure 5).

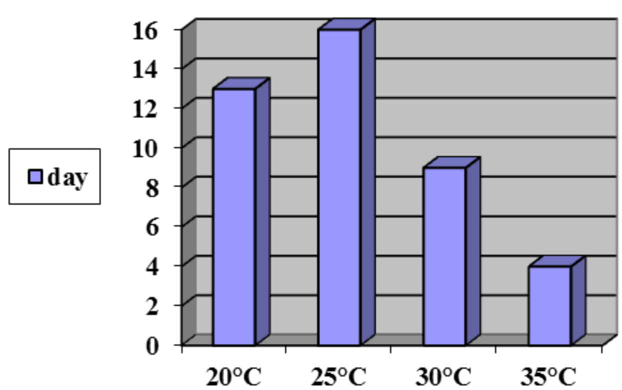

Figure 5) Lifespan of adult flies

Discussion

According to the results of a study, the growth and reproduction of black soldier flies, their growth rate, and seasonal and daily cycles were significantly influenced by temperature (41). It was revealed that temperature affected the biological factors of insects, such as immature survival and adult lifespan, growth, fertility, gender ratio, and population growth parameters (42, 43). Therefore, temperature was found to be one of the effective factors on the life cycle of this insect .Information about the life of this insect can provide knowledge about the most effective changes in the population growth and several other important aspects of the life cycle of this insect that are temperature-dependent (44). Consequently, information on population structure plays a crucial role in achieving the desired temperature, which facilitates the mass production of this species.

Tomberlin et al. (2009) investigated the relationship between temperature and the development of black soldier flies fed on a grain diet at 27, 30, and 36°C. According to the results of the mentioned study, the survival percentage of 4-6-day-old larvae and adults was obtained as 74% and 97% at 27°C and 30°C, respectively, while only 0.1% of the larvae could survive at 36°C. It was also revealed that flies needed an average of 4 more days (11%) to complete larvae and pupae stages at 27°C than at 30°C (45). In another research, Shumo et al. (2019) examined the effect of temperature on selected life-history traits of black soldier fly on two common urban organic waste streams. Accordingly, the optimal temperature range was estimated at 25-30°C (46).

Conclusion

Based on the results of this study, the growth and reproduction of black soldier flies were significantly affected by temperature. The optimum temperature in the breeding of such flies was reported to be 30°C. It was also revealed that temperature affected the insect's biological life cycle, such as immaturity survival, and adult lifespan, growth, fertility, gender ratio, and population growth parameters. The completion of information regarding the factors affecting the life of this insect can provide useful information to its breeders for managing the mass production of this insect. Since this insect provides unique benefits in the promotion of global health, it is recommended to complete information about the factors affecting its life. As a result, helpful information would be available for its breeders regarding the management of this unique species.

Footnotes

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Conflict of Interest

The authors declare that there is no conflict of interest.

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