Influence of Temperature and Humidity on the Development of Tenebrio molitor L.

D. A. Mirzaeva¹, N. A. Khujamshukurov¹*, B. Zokirov¹, B. O. Soxibov² and D. Kh. Kuchkarova³

¹Tashkent Chemical-Technological Institute, ²Surkhandarya Regional Center for Education and Training of Public Education Workers, ³Tashkent Architectural-Civil Engineering Institute, Uzbekistan

*Corresponding author

Abstract

This article determines the effect of temperature on humidity on the development of larvae, pupae and adults of different ages based on the sixth generation (F₆) of the edible insect Tenebrio molitor L. (Coleoptera: Tenebrionidae). The survival properties of young (<30 mg) and adult (<100 mg) larvae, pupae and adults of Tenebrio molitor (F₆) under the influence of relative humidity (RH) of 25, 50, 75 and 95% at a temperature of 10, 25, 35 and 40°C, respectively, for 6 to 48 hours. For moderate survival of young larvae (<30 mg) Tenebrio molitor (F₆), relative humidity 50-75% (survival 92-99%) at 10°C, relative humidity 50-75% (survival rate 92-98%) at a temperature of 35°C is considered a favorable condition, while at a temperature of 40°C and a relative humidity of 25-95% for 12-48 hours showed 100% viability for 6 hours. All other relative humidity showed a sharp loss of viability at this temperature (viability from 76% to 38%). It was found that for adult larvae (<100 mg) Tenebrio molitor (F₆), a relative humidity of 25-75% is convenient at a temperature of 25°C (viability of 100%). Adult larvae (<100 mg) of Tenebrio molitor (F₆) showed moderate relative humidity of 50–75% (viability 98–99%) at a temperature of 10°C for 6-48 h. At a relative humidity of 50%, the average survival periods of time was 95%, and with a relative humidity of 75%, the survival rate of 98.5% that is 3.5% more. It was noted that for the survival of adult larvae (<100 mg) Tenebrio molitor (F₆), a relative humidity below 50% was not suitable. It was noted that the average survival rate for all segments of relative humidity after 12 hours was 92%. It was noted that for the survival of adult larvae (<100 mg) Tenebrio molitor (F₆), a temperature of 40°C and a relative humidity of 25–95% give a stress effect (survival rate of 32%, 44%, 63%, 77%, respectively).

Keywords
Coleoptera, Tenebrionidae, Tenebrio molitor, edible insects, larvae, pupae, adult, insects protein, relative humidity, temperature

Introduction

By 2050, the number of people in the world is expected to exceed 9 billion (Grafton et al., 2015; Park and Yun, 2018). Given the existing production capacities, agroecological situation and the existing agricultural potential, it is impossible to provide a
sufficient number of nutritious products to a sufficient number of people (Dobermann et al., 2017).

In addition, due to abrupt changes in climatic conditions, problems in food production are growing day by day (van Huis and Oonincx, 2017). This requires a search for alternative sources of traditional foods and their in-depth scientific and practical research (Patel et al., 2019). Edible insects can serve as one of such alternative sources (Murefu et al., 2019), (Zielinska et al., 2018). As a result of ongoing research, standard technologies are being developed for the production of products based on edible insects, which are of high nutritional value and are intended to be added to dietary products (Gao et al., 2018).

Due to the rapid development of animal husbandry, poultry farming and fishing, which are among the priorities in feed production, the problem of providing these industries with uninterrupted and nutritious feed products is growing rapidly (Khujamshukurov, 2011). In recent years, the production of feed additives based on edible insects has become one of the most common methods in agriculture. According to Van Huis (2013), mixed locust-based feed grown under controlled conditions (Acheta domesticus) was twice as effective in chickens, four times in pigs, and more than 12 times in cattle.

The feed insect breeding process is characterized by very low environmental damage compared to other types of production, including very low NH₃ and greenhouse gas emissions compared to cattle breeding (Oonincx et al., 2010). In addition, to obtain the protein consumed on the basis of 1 kg of beet flour, very few land areas are required for the production process compared to the land needed for raising chickens, pigs or cattle, and therefore emits very few greenhouse gases into the environment (Oonincx & de Boer, 2012).

In addition to its low environmental impact, it is characterized by a very high level of productivity and the ability to organize the production process regardless of the time of year. In particular, cattle or pigs cannot feed on any plant matter; many types of plants can be used to grow insects.

This also leads to a sharp reduction in the feed problem during cultivation (Durst & Shono, 2010) and further reduces production costs (Mirzaeva et al., 2020a). In world practice, it is noted that there is a wide range of insect species, such as Hermetia illucens, Tenebrio molitor, Acheta domesticus, Alphitobius diaperinus, Zophobas morio, which can be widely used as food and feed additives (FAO, 2014).

One of the main tasks is to create an inexpensive and cost-effective feed base with full nutritional value for raising animals, poultry and eggs, fish and fish products for human consumption, as well as the introduction of technologies for their effective use. In particular, the problem of providing the fish and poultry processing industry in the Republic of Uzbekistan with inexpensive and energy-efficient feed products remains urgent. This is evident from the fact that only in the second half of 2017 and the first half of 2018, the state allocated 630 thousand tons of wheat to the production of feed for the production of livestock products. However, directing this amount of wheat to feed for humans, it is possible to replace it based on non-traditional insect feed biomass (Khujamshukurov et al., 2016; Mirzaeva et al., 2020b).

This study aims to provide people with environmentally friendly feed products with full nutritional value for scientists around the world.
### Table 1: Widely studied insect species as edible insects

| Insect species                        | Protein (%) | Aminoacid (%) | CrudeFibre (%) | NDF(%) | ADF(%) | Minerals(%) | Scientific sources                  |
|---------------------------------------|-------------|---------------|----------------|--------|--------|-------------|--------------------------------------|
| **Lepidoptera**                       |             |               |                |        |        |             |                                      |
| Achroia grisella(L)                   | 33.97       | 60.0          | na             | 19.5   | 8.19   | 1.4         | Finke, 2002                          |
| Bombyx mori(L)                        | 53.75       | 8.09          | na             | 6.36   | 6.36   | 6.36        | Finke, 2002; Frye et al., 1989       |
| Chilcomadia moorei(L)                 | 38.94       | 73.86         | na             | 6.53   | 3.52   | 2.01        | Finke, 2012                          |
| Hyalophora cecropia(L)                | 54.7        | 10.2          | 14.7           | na     | na     | 5.9         | Landry et al., 1986                  |
| Collosamia promethean(L)              | 49.4        | 10.0          | 10.8           | na     | na     | 6.9         | Landry et al., 1986                  |
| Manduca sexta(L)                      | 58.1        | 20.7          | 9.4            | na     | na     | 7.4         | Landry et al., 1986                  |
| Spodoptera frugiperda(L)              | 57.8        | 20.2          | 6.7            | na     | na     | 5.6         | Landry et al., 1986                  |
| Pseudaletia unipuncta(L)              | 54.4        | 14.9          | 5.0            | na     | na     | 6.9         | Landry et al., 1986                  |
| Spodoptera eridania(L)                | 54.7        | 13.9          | 7.1            | na     | na     | 9.8         | Landry et al., 1986                  |
| Samia ricini(P)                       | 54.2        | 26.20         | 3.26           | na     | na     | 3.80        | Longvah et al., 2011                 |
| Samia ricinii(P)                      | 54.6        | 26.20         | 3.45           | na     | na     | 3.80        | Longvah et al., 2011                 |
| Cirina forda                          | 20.0        | 12.5          | 8.7            | na     | na     | NA          | Osasona & Olaofe, 2010               |
| Antheraea pernyi                      | 71.9        | 20.1          | na             | na     | na     | 4.0         | Zhou & Han, 2006                     |
| Z.morii(A)                            | 68.05       | 14.25         | na             | 50.14  | 32.06  | 6.16        | Oonincx & Dierenfeld, 2012           |
| Z.morio                               | 46.79       | 42.04         | na             | 9.26   | 6.41   | 2.38        | Finke, 2002                          |
| T.molitor                            | 49.08       | 35.17         | 14.96          | na     | 6.56   | 2.36        | Finke, 2002                          |
| Cotinis nitida                       | 51.75       | 5.41          | 19.3           | na     | na     | 12.34       | Rashashantong et al., 2010           |
| **Hymenoptera**                      |             |               |                |        |        |             |                                      |
| Oecophyllas maragdina                 | 53.46       | 13.46         | 15.38          | na     | na     | 6.55        | Rashashantong et al., 2010           |
| **Orthoptera**                       |             |               |                |        |        |             |                                      |
| Acheta domesticus                     | 66.56       | 22.08         | na             | 22.08  | 10.39  | 3.57        | Finke, 2002; Bernard et al., 1997    |
| Microcentrum hombifolium(A)           | 77.80       | 9.00          | na             | 41.14  | 19.39  | 9.10        | Oonincx & Dierenfeld, 2012           |
| Anurogryllus arboreus                 | 48.69       | 20.60         | 11.61          | na     | na     | 9.36        | Rashashantong et al., 2010           |
| **Diptera**                           |             |               |                |        |        |             |                                      |
| H.illucens                            | 45.10       | 36.08         | na             | 9.79   | 7.73   | 9.02        | Finke, 2012                          |
| Musca domestica(L)                    | 78.17       | 7.5           | na             | 14.29  | 11.51  | 6.75        | Finke, 2012                          |
| D.melanogaster(A)                     | 68.00       | 19.00         | na             | 17.66  | 10.14  | 7.20        | Oonincx & Dierenfeld, 2012; Barker et al., 1998 |
| **Blattodea**                         |             |               |                |        |        |             |                                      |
| Blatta Lateralis                      | 61.5        | 32.4          | na             | 9.06   | 7.12   | 3.9         | Finke, 2012                          |
| B.lateralis(S)                        | 76.05       | 14.45         | na             | 11.41  | 10.87  | 7.88        | Oonincx & Dierenfeld, 2012           |
| B.Lateralis(M)                        | 62.85       | 26.50         | na             | 12.76  | 12.75  | 6.89        | Oonincx & Dierenfeld, 2012           |
| Eublaberus distanti                   | 52.1        | 43.1          | na             | na     | na     | 2.98        | Oonincx & Dierenfeld, 2012           |
| Gromphador hinaportentosa             | 63.35       | 20.30         | na             | 36.54  | 13.12  | 8.49        | Oonincx & Dierenfeld, 2012           |
| Periplaneta americana                 | 53.9        | 28.4          | na             | na     | 9.4    | 3.3         | Bernard et al., 1997                 |

A-Adult, M-medium, S-small, L-larvae, P-pupae, PP-prepupae, na-not applicable
To solve this problem, scientists and specialists all over the world have introduced many methods and technologies. One of the most convenient and inexpensive alternatives is to grow these edible insects and use them as food and feed. Table 1 shows the protein and amino acid compositions that determine the nutritional value of some insectivores reflected in scientific sources.

One of the most promising areas of modern production is the development of feed products based on edible insects that are easy to store in terms of high protein content and low fat content and easy digestion.

In Uzbekistan, the production and use of feed products and additives based on edible insects has not yet been implemented. However, global insect-based economic growth in 2019 reached $112 million and is projected to increase by another 47% in 2019–2026 (Industry Forecasts, 2020).

At the end of 2019, the economic indicator in the general edible insect market amounted to $24.18 million, while the share of European countries amounted to $10.34 million (Kunal Ahuja and Kritika Mamtani, 2020). This indicates that there is competition for the further development of stable economic indicators in the edible insect market. Therefore, one of the most promising areas in Uzbekistan is the cultivation of edible insects in controlled conditions, the introduction of practices based on their use in animal husbandry, poultry farming and fishing.

The aim of this study was to study the effect of temperature with relative humidity on the development of the larvae, pupae, and adults of the edible insect Tenebrio molitor L. (Coleoptera: Tenebrionidae).

### Table 2

| Type of insects | The scientific name of the insect | Protein | Fat | Scientific sources            |
|-----------------|----------------------------------|---------|-----|-------------------------------|
| Larvae          | Allomyrina dichotoma             | 54.18   | 20.24 | Ghosh et al., (2017)          |
|                 | Anaphe infracta                  | 20.00   | 15.20 | Banjo et al., (2006)          |
|                 | Anaphe recticulata               | 23.00   | 10.20 | Banjo et al., (2006)          |
|                 | Anaphe venata                    | 25.70   | 23.21 | Banjo et al., (2006)          |
|                 | Gonimbrasia belina               | 56.95   | 10.00 | Siulapwa et al., (2014)       |
|                 | Gynani samaja                    | 55.92   | 12.10 | Siulapwa et al., (2014)       |
|                 | Protoaetia brevitarsis           | 44.23   | 15.36 | Ghosh et al., (2017)          |
|                 | Rhynchophorus phoenicis          | 22.06   | 66.61 | EkpoandOnigbinde (2005)       |
|                 | Tenebrio molitor                 | 46.44   | 32.70 | Ravzanaadii et al., (2012)    |
| Beetle          | Heteroligus meles                | 38.10   | 32.01 | Jonathan (2012)               |
|                 | Oryctes boas                     | 26.00   | 1.50  | Banjo et al., (2006)          |
|                 | Rhynchophorus phoenicis          | 50.01   | 21.12 | Jonathan (2012)               |
|                 | Rhynchophorus phoenicis          | 28.42   | 31.40 | Banjo et al., (2006)          |
| Grasshopper     | Ruspolia differens               | 44.59   | 49.00 | Siulapwa et al., (2014)       |
|                 | Zonocerus variegatus             | 26.80   | 3.80  | Banjo et al., (2006)          |
| Cricket         | Brachytrypes spp.                | 6.25    | 2.34  | Banjo et al., (2006)          |
|                 | Gryllus bimaculatus              | 58.32   | 11.88 | Ghosh et al., (2017)          |
|                 | Teleogryllus emma                | 55.65   | 25.14 | Ghosh et al., (2017)          |
| Termites        | Macrotermes bellicosus           | 20.10   | 28.20 | Banjo et al., (2006)          |
|                 | Macrotermes falciger             | 43.26   | 43.00 | Siulapwa et al., (2014)       |
|                 | Macrotermes notalensis           | 22.10   | 22.50 | Banjo et al., (2006)          |
| Bee             | Apis mellifera                   | 21.00   | 12.30 | Banjo et al., (2006)          |
| Dragonfly       | Aeschna multicolor              | 54.24   | 16.72 | Ramos-Elorduy et al., (1998)  |
|                 | Anax sp.                         | 26.22   | 22.93 | Ramos-Elorduy et al., (1998)  |
Materials and Methods

The sixth generation (F₆) *Tenebrio molitor* (*Coleoptera: Tenebrionidae*), collected from the southern foothills of Uzbekistan and propagated under controlled conditions, was obtained on the basis of variant F₁. In this study, variants of larvae of TMO-2 and TMO-6 based on variant F₆ were used to determine viability. During the study, 2 larvae (F₁ variant) were selected from the collected and numbered *Tenebrio molitor* larvae and beetles (128: larvae 108, beetles 20), which were the largest in size (TMO-2: 5.36 cm, TMO-6: 4.83 cm) compared with the rest during visual observation, a *Tenebrio molitor* colony was formed on their basis. Growing conditions: Used a special nutrient medium based on wheat bran, *Lemna minor* and *Azollacarolina*, developed by the authors. Constant insect retention temperature 20-22°C. The studies used temperatures of 10-40°C. Lighting mode (photoperiodic mode): 8 hours of light, 16 hours of darkness. In studies, the age of the larvae was divided based on accurate measurements. Young larvae <30 mg, adult larvae <100 mg. The larvae, cocoons, and beetles of the objects of study were formed in a colony of 10 groups each. The number of objects in each group was set to 30. (RH) - relative humidity is calculated in %.

Relative humidity was calculated using the following formula: \( \varphi = \frac{\rho - \rho_H}{\rho_H} \times 100\% \). Here, \( \rho \) - water vapor density (absolute humidity), kg/m² (SI system) or g/m³; \( \rho_H \) - density of saturated water vapor at a certain temperature, kg/m³ or g/m³. A psychrometer was used to determine relative humidity. A standard scale on a psychrometer was used as a relative humidity scale.

To maintain constant humidity, the containers in which insect larvae, cocoons and beetles were grown were hermetically closed (30 min), a vessel for generating steam was inserted into the chamber, a moist chamber was formed, and the specified humidity was maintained. The study used the Bloomberg method to ensure that the temperature and relative humidity in each container were the same (for example, 25°C/75% per hour) (Blumberg, 1971).

The calculation of statistical error, mean value, confidence intervals and standard deviations of experimental data was carried out using the computer program STATISTICA 6.0 and standard methods. The statistical significance of the results was determined using Student's t-criterion.

Results and Discussion

The survival characteristics of the *Tenebrio molitor* beetle, which is considered aedible insect, grew at different temperatures and relative humidity, and the dynamics of its development were studied. The results showed that young larvae weighing about 30 mg showed a survival rate of 58% when they were kept at 25% relative humidity for 6 hours at 10°C, and a survival rate of 92-99% when they were kept at a relative humidity of 50-75% (fig. 1).

While maintaining a relative humidity of 95% for 6 hours, there is a sharp decrease in survival to 78%. When the larvae were kept at 10°C for 12 hours at 25% relative humidity for 25 hours at 25-50% relative humidity, the survival rate was 52-83%, at 75% relative humidity the survival rate was 99%, at 95% relative humidity the survival rate was sharp fell 49%, respectively.

The larvae showed a viability of 29% at a relative humidity of 25%, 68% at a relative humidity of 50%, and 77% at a relative humidity of 75% when kept for 24 hours, which indicates that the indicators moved from bottom to top. It was noted that in larvae captured within 24 hours at a relative humidity
of 95%, viability was sharply reduced to 34%. Young larvae weighing about 30 mg showed very low viability - 9% at a relative humidity of 25%, while at a relative humidity of 50-75%, the survival of these larvae increased to 52-63%. The survival rate of the above larvae in this case did not show the survival rate of 11% of larvae that remained for 48 hours at 95% humidity. Analyzing the statistical data, it was noted that at a temperature of 10°C with a relative humidity of 50-75% at all time periods, the humidity was moderate for young (<30 mg) Tenebrio molitor larvae. At a relative humidity of 50%, the average survival rate was 73.75%, and at a relative humidity of 75% - 84.5%, that is, 10.75% higher.

Figure 2 shows the survival of adult larvae (<100 mg) of Tenebrio molitor (F_6) at 10°C and various relative humidity. The results showed that adult larvae showed a viability of 98-99% when kept at all values of relative humidity for 6-12 hours. However, at this temperature, adult larvae showed a viability of 98% with a relative humidity of only 50–75% for 24 hours, a viability of 79% with a humidity of 25% and 73% with a relative humidity of 95%.

A similar trend was observed in larvae recorded at a relative humidity of 48–95% for 48 h, with viability 68–70%, respectively. In adult larvae kept at a relative humidity of 50–75% for 48 hours, a corresponding survival rate of 84–98% was observed. At a temperature of 10°C, relative humidity of 50–75% in all time intervals was moderate humidity for young (<100 mg) Tenebrio molitor larvae. At a relative humidity of 50%, the average survival time was 95%, and at a relative humidity of 75%, the survival rate was 98.5%, that is, 3.5% more. Compared to young larvae (<30 mg), retained at a relative humidity of 50–75% at 10°C, in adult larvae (<100 mg), the average survival over time periods was 21.25% at a relative humidity of 50% and at a relative humidity 75% showed a high survival rate of 14.0%. This indicates that adult larvae are more resistant to this relative humidity than young larvae.

In fig. Figure 3 shows the data obtained by growing Tenebrio molitor pupae at different relative humidity and at a temperature of 10°C. According to the results, when the pupae were kept for 6-12 hours at a relative humidity of 25-95%, their average survival rate was 98.75%. When the pupae were kept at 25% relative humidity for 24 hours, 86% and at 95% relative humidity, the survival rate was 78%. It was noted that the average survival rate was 98% when the pupa was kept at a relative humidity of 50-75% for 24 hours. It was observed that pupae kept at a relative humidity of 25% for 48 hours showed a viability of 86%, while survival at a relative humidity of 50% decreased to 82%. However, pupae kept at a relative humidity of 75% over the same period of time showed a viability of 98%. At a relative humidity of 95%, a viability of 74% was observed. For 6–12 h, the average survival of pupae at a relative humidity of 25–95% was 98.75%, and the average survival of pupae at a relative humidity of 25–95% for 24 h was 91.5%, and the average survival of pupae held at the relative humidity of 25-95% for 48 hours was 85.05%, while the survival rate was 6.5% higher after 24 hours than after 48 hours.

The average survival of pupae at a relative humidity of 25–95% for 12–24 h was 98.75%, while the average survival was 10.5% higher than that of pupae that remained for 24–48 h at this humidity.

Figure 4 shows the survival rates of adult Tenebrio molitor (F_6) individuals at a temperature of 10°C and various relative humidity. It was found that in adults the overall survival rate is 98.5% when stored at a relative humidity of 25–95% at 10–12°C for 6–12 hours.
Adults held at these relative humidity levels for 24 hours showed an average survival rate of 32.1%, while adults held at 48 hours showed an average survival rate of 49.75%. It was noted that the average adult survival for 24-48 hours at a relative humidity of 25-95% was 57%, and the survival rate was 41.5% lower than that for adults held for 6-12 hours at a relative humidity of 25-95%.

Figure 5 shows the survival of young *Tenebrio molitor* (F₀) larvae (<30 mg) at 25°C and various relative humidity. In accordance with the results, it was noted that all young larvae stored at 25°C for 6-24 hours at a relative humidity of 25-75% showed 100% viability. Larvae kept at a relative humidity of 25% for 48 hours showed a viability of 90%. Larvae retained at a relative humidity of 95% for 6-12 h also showed 100% viability, while larvae kept at this relative humidity for 48 h showed 92% viability. The results showed that the optimum temperature for the moderate development of young larvae is humidity in the range of 25°C and relative humidity of 50-75%.

Figure 6 shows the survival of large larvae (<100 mg) of *Tenebrio molitor* (F₀) at 25°C and various relative humidity. According to the results of the study, all adult larvae showed 100% viability at a temperature of 25°C and a relative humidity of 25-95%. Similar indicators were observed in pupae (Fig. 7) and adults (Fig. 8) *Tenebrio molitor*.

Figure 9 shows the survival of young larvae (<30 mg) of *Tenebrio molitor* (F₀) at 35°C and various relative humidity. According to the results, young larvae showed a viability of 71% when stored at 25% relative humidity for 6 hours at 35°C, 92-98% at a relative humidity of 50-75% and 61% at a relative humidity of 95%. At this time, the average survival at all relative humidity was 81.5%. It was found that young larvae at 12% relative humidity for 12 hours had a survival rate of 61%, at 82% relative humidity 50%, at 92% relative humidity 75% and at 35% relative humidity 95%.

When larvae were stored for 24 and 48 hours, these indicators tended to decrease sharply. In particular, it was found that the average survival in all segments of moisture after 24 hours was 47%, and when stored for 48 hours - 28%. Figure 10 shows the survival of large larvae (<100 mg) of *Tenebrio molitor* (F₀) at 35°C and various relative humidity. According to the results, adult larvae, stored for 6 hours at a relative humidity of 25-95%, showed 100% viability. It was found that the larvae stored at a relative humidity of 25% for 12 h had a viability of 92%, at 50-75% relative humidity of 100% and at 95% relative humidity of 78%.

It was noted that the average survival rate for all segments of relative humidity after 12 hours was 92%. Adult larvae stored at 25% relative humidity for 24 hours showed a viability of 65%, at 50% relative humidity 85% and at 75% relative humidity 100%. Over a 24-hour period, the average survival of adult larvae in all segments of moisture was 69.5%. When adult larvae were kept at a relative humidity of 95% for 48 hours, survival was sharply reduced to 49%.

In this case, the overall survival of adult larvae retained at a relative humidity of 95% for 48 hours averaged 50.25%. Figure 11 shows the survival of *Tenebrio molitor* (F₀) pupae at 35°C and various relative humidity. According to the results, the pupae showed 100% viability when stored at 35°C and at 25-50% relative humidity for 6-12 hours. Although pupae stored for 24 hours at 25% relative humidity showed a viability of 82%, they showed 100% viability when stored for 24-7 hours at a relative humidity of 50-75%. However, observations showed that while maintaining a relative humidity of 95% for 48 hours, it loses viability by 44.5%.
According to these results, it was determined that the average relative humidity should be 50-75%. This is also evidenced by the fact that pupae stored for 48 hours at a relative humidity of 25% lose 28% viability, at 50% relative humidity 18% and at 75% relative humidity 8%.

It was also noted that at 95% relative humidity, a moisture loss of 81%. Figure 12 shows the survival rates of adult *Tenebrio molitor* (F₆) individuals at a temperature of 35°C and various relative humidity, while adult individuals demonstrate 100% viability when stored at 25–95% relative humidity for 6–48 hours.

It was also noted that adults held for 12 hours at 50–75% relative humidity also showed 100% survival. It was found that adults lost 18% viability when storage was 6 hours at 25% relative humidity, and adults lost 91% survival when stored for 48 hours at 95% relative humidity. Therefore, in this case, evaporation occurs together with the temperature of relative humidity, free oxygen is lost, which leads to the death of the insect due to breathing difficulties.

This situation can also be observed in the survival rates of young larvae (<30 mg) of *Tenebrio molitor* (F₆), shown in Figure 13, at a temperature of 40°C and various relative humidity. In particular, it was noted that young larvae, which showed 100% viability in all cases of observation, lost 38, 42, 58, and 76% viability when stored at 40°C and at 255% relative humidity for 6-48 hours, respectively. In proportion to this, a sharp loss of viability was observed at any relative humidity and storage time. Thus, it was noted that all relative humidity at 40°C negatively affects young larvae. Adult larvae, on the other hand, can be resistant to a relative humidity of 25-50% for 6 hours (Fig. 14). In particular, a 75% survival rate was observed at a relative humidity of 25% and a survival rate of 68% at a relative humidity of 50%. However, it was reported that adult larvae that were stored for 6 hours at 75% and 95% relative humidity lost 62–78% viability, respectively. It was noted that at a temperature of 40°C for 12 hours at 25-95% relative humidity, 32, 44, 63 and 77%, respectively, are lost.

When stored at these relative humidity levels for 24 and 48 hours, a stressful condition occurred with a loss of viability of 58–77, 61–82, 72–89, and 84–92%, respectively. Therefore, for adult larvae, a temperature of 40°C and a relative humidity of 50-95% are one of the stress factors. Figure 15 shows the survival rates of *Tenebrio molitor* (F₆) pupae at 40°C and various relative humidity. The results showed that pupae lost an average of 38-32% viability when stored at 25-50% relative humidity for 6 hours, respectively, while when stored for 12 hours at this relative humidity, they lost 51-48% viability.

It was also found that a sharp loss of viability was observed under storage conditions for 24–48 h at relative humidity. In particular, it was found that pupae lose 89, 92, 94, and 96% viability, respectively, when stored in the studied humidity for 6–48 hours.

It has been reported that a temperature of 40°C has a sharp negative effect on adult survival of *Tenebrio molitor* (F₆) (Fig. 16). In particular, adults who stayed for 6 hours at a relative humidity of 25-95% lost viability of 36, 42, 48, and 74%, respectively, while survival rates of 44, 54, 60, and 72% were recorded for 12 hours at these relative humidity levels, respectively.

The lowest rate was observed at 48 hours of storage. In particular, a viability of 88, 86, 91, and 96% was detected at a relative humidity of 25-95%, respectively. This indicates that a temperature of 40°C is also one of the stress factors in adult survival.
Figure 1. Survival rates of young larvae of *Tenebrio molitor* (F6) (<30 mg) at 10°C and different relative humidity, %

Figure 2. Survival rates of large larvae of *Tenebrio molitor* (F6) (<100 mg) at 10°C and different relative humidity, %

Figure 3. *Tenebrio molitor* (F6) pupae survival rates at 10°C temperature and different relative humidity, %
Figure 4 *Tenebrio molitor* (F₆) survival rates of adults at 10°C temperature and different relative humidity, %

Figure 5 Survival rates of young larvae of *Tenebrio molitor* (F₆) (<30 mg) at 25°C and different relative humidity, %

Figure 6 Survival rates of large larvae of *Tenebrio molitor* (F₆) (<100 mg) at 25°C and different relative humidity, %

Similar indicators were observed in pupae (Fig. 7) and adults (Fig. 8) *Tenebrio molitor*.

Figure 7 *Tenebrio molitor* (F₆) pupae survival rates at 25°C temperature and different relative humidity, %
Figure 8 Tenebrio molitor (F_6) adults' survival rates at 25°C and various relative humidity, %

Figure 9 The survival rates of young larvae of Tenebrio molitor (F_6) (<30 mg) at 35°C and different relative humidity, %

Figure 10 Survival rates of large larvae of Tenebrio molitor (F_6) (<100 mg) at 35°C and different relative humidity, %

Figure 11 Tenebrio molitor (F_6) pupae survival rates at 35°C temperature and different relative humidity, %
Figure 12: *Tenebrio molitor* (F6) adults' survival rates at 35°C and various relative humidity, %

Figure 13: Survival rates of young larvae (<30 mg) of *Tenebrio molitor* (F6) at 40°C and different relative humidity, %

Figure 14: Survival rates of large larvae (<100 mg) of *Tenebrio molitor* (F6) at 40°C and different relative humidity, %

Figure 15: Survival rates of *Tenebrio molitor* (F6) pupae at 40°C temperature and various relative humidity, %
It is known that the use of specified technological parameters when growing edible insects under controlled conditions leads to economic efficiency. Technological indicators widely used in world practice, the specific climatic conditions of each region, the specificity of the *Tenebrio molitor* beetles and, of course, the nature of variability depending on the knowledge and skills of specialists. In particular, the minimum and maximum temperatures for *Tenebrio molitor* pupae were defined as 10°C (Punzo and Mutchmor, 1980), 15°C (Kim et al., 2015), 17°C (Koo et al., 2013), 20°C (Martin et al., 1976), given that the optimum temperature is about 25-27°C (Siemianowska et al., 2013), 27-28°C (Kim et al., 2015; Koo et al., 2013; Spencer and Spencer, 2006), 27-33°C (Hardouin and Mahoux, 2003) and a maximum temperature of 30°C (Manoilovich, 1987; Koo et al., 2013), 35°C (Kim et al., 2015; Punzo and Muchmore, 1980). Also, relative humidity for moderate development of larvae is at least 12% (Punzo, 1975; Punzo and Mutchmor, 1980), 30% (Fraenkel, 1950), 60-70% as optimal relative humidity (Spencer and Spencer, 2006), 70% (Hardouin and Mahoux, 2003), 75% (Punzo, 1975; Punzo and Mutchmor, 1980), a maximum of 98% (Punzo, 1975; Punzo and Muchmore, 1980). A minimum of 12% for the development of pupae depending on relative humidity (Punzo, 1975; Punzo and Mutchmor, 1980), the optimal 70% (Murray, 1968) 75% (Punzo, 1975; Punzo and Mutchmor, 1980) and a maximum of 98 (Punzo, 1975; Punzo and Muchmore, 1980).

The minimum relative humidity of beetles is 12% (Punzo, 1975; Punzo and Mutchmor, 1980), 20% (Dick, 2008; Hardouin and Mahoux, 2003), the optimal 70% (Murray, 1968), 75% (Punzo, 1975; Punzo and Mutchmor, 1980), 90–100% (Hardouin and Mahoux, 2003), 98% as maximum relative humidity (Punzo, 1975; Punzo and Muchmore, 1980).

Analyzing scientific sources, one can see that in all cases there are sharply different indicators, and these technological indicators cannot be realized without research.

In our study, the total protein and amino acid composition of natural F1 variants of the *Tenebrio molitor* L. beetles (Mirzaeva et al., 2020a), the survival of the F6 generation variants in wheat bran and macrophytes (*Lemma minor* and *Azollacarolina*) based on variant F1, we studied such features as the rate of oviposition (Mirzaeva et al, 2020b). The results of these studies on the survival of *Tenebrio molitor* larvae, pupae and adults at relative humidity depending on temperature make it possible to establish technological parameters for growing edible insects under controlled conditions. This will allow in the Republic of Uzbekistan to master the technology of controlled feeding of insects (Khujamshukurov, 2011), which has not yet been put into practice in local conditions. This serves as an unconventional source of enrichment for the nutritious and uninterrupted feed supply of livestock, poultry, and fishing in the country (Khujamshukurov et al., 2016).
Acknowledgement

This study was carried out as part of an innovative project of the Ministry of Innovative Development of the Republic of Uzbekistan No. I-OT-2019-21 and conducted as part of the scientific program of the Tashkent Institute of Chemical Technology "Development of feeding technology based on non-traditional sources". We express our gratitude to the staff of the head scientific laboratory "Biotechnology" (prof. N.A. Khujamshukurov) of the Tashkent Institute of Chemical Technology.

References

Banjo AD., LawalOA.,Songonuga EA. 2006. The nutritional value of fourteen species of edible insects in southwestern Nigeria. Afr. J. Biotechnol 5:298-301.

Barker D., Fitzpatrick M.P., Dierenfeld E. S. 1998. Nutrient composition of selected whole invertebrates. ZooBiol. 17(2): 123-134.

Bernard J.B., Allen M.E., Ullrey D.E. 1997. Feeding captive insectivorous animals: Nutritional aspects of insects as food. NutritionAdvisoryGroupHandbook, FactSheet. 3: 1-7.

Blumberg D. 1971. Survival capacity of two species of Cybocephalus (Coleoptera: Cybocephalidae) under temperature and humidity extremes. Ent. Exp. Appl. 14: 433-440.

Dick J. 2008. Oviposition in Certain Coleoptera. Ann. Appl. Biol. 24, 762–796. doi:10.1111/j.1744-7348.1937.tb05055.x.

Dobermann D., Swift JA., Field LM. 2017. Opportunities and hurdles of edible insects for food and feed. Nutr Bull 42:293308.

Ekpo KE., Onigbinde AO. 2005. Nutritional potentials of the larva of Rhynchophorus phoenicis (F). Pak J Nutr 4:287-290.

FAO. 2014. The State of World Fisheries and Aquaculture. Opportunities and challenges. Food and agriculture organization of the United Nations, Rome. P.243.

Finke M.D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. ZooBiol. 21(3): 269-285.

Finke M.D. 2012. Complete nutrient content of four species of feeder insects. ZooBiol. 00:1-15.

Fraenkel G., 1950. The Nutrition of the Mealworm, Tenebrio molitor L. (Tenebrionidae, Coleoptera). Physiol. Zool. 23, 92–108. doi:10.1017/CBO9781107415324.004.

Frye F.L., Calvert C.C. 1989. Preliminary information on the nutritional content of mulberry silk moth (Bombyx mori) larvae. J. Zoo. Wildl. Med. 20: 73-75.

Gao Y., Wang D., Xu M.L., Shi S.S., Xiong J.F. 2018. Toxicological characteristics of edible insects in China: A historical review. Food ChemToxicol 119:237-251.

Ghosh S., Lee S.M., Jung C., Meyer-Rochow VB. 2017. Nutritional composition of five commercial edible insects in South Korea. J Asia-PacEntomol 20:686-694.

Grafton RQ, Daugbjerg C, Qureshi ME. 2015. Towards food security by 2050. Food Secur 7:179-183.

Hardouin J., Mahoux G. 2003. Zootechnied’insectes - Elevageetutilisation au bénéfice de l’homme et de certainsanimaux. Bull. Semest. d’InformationsurleMini-ElevageNuméspé, 164.

Industry Forecasts. 2020. Edible Insects Market Analysis 2020-2026. https://www.gminsights.com/industry-analysis/edible-insects-market.

Jonathan AA. 2012. Proximate and anti-nutritional composition of two common
edible insects: Yam beetle (Heteroligusmeles) and palm weevil (Rhynchophorusphoenicis). Food Sci 49:9782-9786.

Khujamshukurov N.A. 2011. Alternative protein products. J. XXI-technology. №4 (5):14-15.

Khujamshukurov N.A., NurmuXamedova V.Z. 2016. Production feed: modern trend and development aspect. Scientificoverview. J.Zooveterinary. №8 (105):34-37.

Kim S.Y., Park J., Bin Lee Y.B., Yoon H.J., Lee K.Y., Kim N.J. 2015. Growthcharacteristics of mealworm Tenebrio molitor L. (Coleoptera: Tenebrionidae). Korean J. Appl. Entomol. 52, 387–394.

KunalAhuja., KritikaMamtani. 2020. Edible Insects Market Size By Product (Beetles, Caterpillars, Grasshoppers, Bees, Wasps, Ants, Scale Insects & Tree Bugs), By Application (Flour, Protein Bars, Snacks), Industry Analysis Report, Regional Outlook, Application Potential, Price Trends, Competitive Market Share & Forecast, 2020-2026. Industry Forecasts. P.170. https://www.giminsights.com/industry-analysis/edible-insects-market

Kurbanov AR., Milusheva RY., Rashidova SS., Kamilov BG. 2015. Effect of replacement of fish meal with silkworm (Bombyxmori) pupa protein on the growth of Clariasgariepinus Fingerling. Int J FishAquatStud 2:25-27.

Landry S.V., DeFoliart G.R., Sunde M.L. 1986. Larval protein quality of six species of Lepidoptera (Saturniidae, Sphingidae, Noctuidae). J. Econ. Entomol. 79, 600-604.

Longyah T., Mangthya K., Ramulu P., 2011. Nutrient composition and protein quality evaluation of eri silkworm (Samiaricinii) prepupae and pupae. FoodChem. 128(2): 400-403.

Martin R.D., Rivers J.P.W., Cowgill U.M. 1976. Culturing mealworms as food for animals in captivity. Int. ZooYearb. 16, 63–70. doi:10.1111/j.1748-1090.1976.tb00130.x.

Mirzaeva D.A., Maksumkhodzhaeva K.S., Khujamshukurov N.A., Abdullaev X.O., GazievaSh.Q., IskhakovaSh.M., KuchkarovaD.Kh. 2020a. Dependence of the food environment on protein synthesis of feed insects. Int. J. Curr. Microbiol. App. Sci. 9(04):2225-2232.

Mirzaeva D.A., Maksumkhodzhaeva K.S., Khujamshukurov N.A., Abdullaev X.O., GazievaSh.Q., IskhakovaSh.X., KuchkarovaD.X. 2020b. Nutrition Value of Lemnaceae Macrophytes. Int. J. Curr. Microbiol. App. Sci. 9(04):3233-3242.

Murefu TR., Macheka L., Musundire R., Manditsera FA. 2019. Safety of wild harvested and reared edible insects: A review. FoodControl 101:209-224.

Murray D.R.P. 1968. The Importance of water in the normal growth of larvae of Tenebrio molitor. Entomol. Exp. Appl. 11, 149-168. doi:10.1017/CBO9781107415324.004

Oonincx D.G.A.B., Dierenfeld E.S. 2012. An investigation into the chemical composition of alternative invertebrate prey. ZooBiol. 31(1): 40-54.

Oonincx D.G.A.B., van der Poel A.F.B.2010. Effects of diet on the chemical composition of migratory locusts (Locustamigratoria). ZooBiol. 28:1-8.

Osasona A.I., Olaofe O. 2010. Nutritional and functional properties of Cirinafortdalarva from Ado-Ekiti, Nigeria. Afr. J. FoodSci. 12(4): 775-777.

Park S., Yun E. 2018. Edible insect food: Current scenario and future perspectives. FoodSciAnimResourInd 7:12-20.

Patel S., Suleria HAR., Rauf A. 2019. Edible
insects as innovative foods: Nutritional and functional assessments. Trends Food Sci Technol 86:352-359.

Punzo F. 1975. Effects of temperature, moisture and thermal acclimation on the biology of Tenebrio molitor (Coleoptera: Tenebrionidae). Iowa State University.

Punzo F., Mutchmor J.A. 1980. Effects of Temperature, Relative Humidity and Period of Exposure on the Survival Capacity of Tenebrio molitor (Coleoptera: Tenebrionidae). J. Kansas Entomol. Soc. 53, 260–270. doi:10.2307/25084029.

Raksakantong P., Meeso M., Kubola J., Siriamornpun S. 2010. Fatty acids and proximate composition of eight Thai edible terricolous insects. Food Research International. 43:350-355.

Ramos-Elorduy J., Pino-M JM., Correa SC. 1998. Edible insects of the state of Mexico and determination of their nutritive values. An.Inst.Biol.Univ.Nac.Auton.Mex.Ser.Zool 69:65-104.

Ravzanaadii N., Kim SH., Choi WH., Hong SJ., Kim NJ. 2012. Nutritional value of mealworm, Tenebrio molitor as food source. Int J Indus Entomol 25:93-98.

Siemianowska E., Kosewska A., Aljewicz M., Skibniewska K.A., Polak-Juszczyk L., Jarocki A., Jędras M. 2013. Larvae of mealworm (Tenebrio molitor L.) as European novel food. Agric. Sci. 4, 287–291. doi:10.4236/as.2013.46041.

Siulapwa N., Mwambungu A., Lungu E., Sichilima W. 2014. Nutritional value of four common edible insects in Zambia. Int J. SciRes 3:876-884.

Spencer W., Spencer J. 2006. Management Guideline Manual for Invertebrate Live Food Species. EAZA Terr. Invertebr. TAG. 1–54.

vanHuis A., Oonincx DGAB. 2017. The environmental sustainability of insects as food and feed. A review. Agron Sustain Dev 37:43.

Zhou J., Han D. 2006. Proximate, amino acid and mineral composition of pupae of the silkworm Antheraea pernyi in China. Journal of Food Composition and Analysis. 19:850-853.

Zielinska E., Karas M., Baraniak B. 2018. Comparison of functional properties of edible insects and protein preparations thereof. LWT-Food Sci Technol 91:168-174.

How to cite this article:

Mirzaeva, D.A., N. A. Khujamshukurov, B. Zokirov, B. O. Soxibov and Kuchkarova, D. Kh. 2020. Influence of Temperature and Humidity on the Development of Tenebrio molitor L. Int.J.Curr.Microbiol.App.Sci. 9(05): 3544-3559. doi: https://doi.org/10.20546/ijemas.2020.905.422