Article

Yellow meal worm (*Tenebrio molitor L.*) development time of life stages duration and survival rate at different temperatures in laboratory conditions

Shokouh Heidari Parsa[1], **Farshid Kheiri**[3], **Yaghoub Fathipour**[2], **Sohrab Imani**[1], **Mohamad Chamani**[4]
[1]Department of Entomology, Faculty of Agricultural and Natural Resources, Tehran Science and Research, Islamic Azad University, Tehran, Iran
[2]Department of Animal Science, Shahrekord Branch ,Islamic Azad University, Shahrekord, Iran
[3]Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
[4]Department of Animal Science, Faculty of Agricultural and National Resources, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran
E-mail: Imanisohrab@gmail.com

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Abstract
The yellow mealworms (*Tenebrio molitor L.*) is one of the most valuable and high-nutritional food insects that is used in the animal and human feed industry. In the current study development time of eggs, larvae, pupa, and the entire immature stage, at five constant temperatures of 20, 23, 25, 27, and 30°C (±1), 60±10% RH was examined under laboratory conditions. The eggs in the same age (less than 24 hours) were randomly selected. The growth was completed at all temperatures of the test. Data showed that the developmental times of males or females on each variety at various constant temperatures differed significantly. The regression and Ikemoto and Takailinear models, in the absence of 27°C described the relationship of developmental rate to temperature for males and females of *T. molitor* very well. Data were fitted to various nonlinear temperature-dependent models. The model of Lactin-1 and Janisch/Rochat have the best estimate for T_u about 36.79 and 37.36°C. Development time of immature stage was 203.70±2.65, 170.71±3.51, 143.07±2.29, 111.31±0.81 day. The longest development time was recorded at 20°C and the shortest growth period at 27°C. The results of the variance of the data showed that the development time of the immature stages at different temperatures at a 5% probability level was significant. Although by the increasing in temperature from 20 to 27 development time was declined but that was not associated with increased growth rates.

Keywords yellow meal worm; temperature; development time; linear and non-linear model.

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1 Introduction

The yellow mealworm (*Tenebrio molitor* L.) is one of the insects that infest stored cereal products (Back and Cotton, 1922) (Bjorge et al., 2018). The larvae of this insect molt several times (9-23) with an increase of their size, their color changes from white to yellow (Cotton, 1927; Hill, 2002; Ludwig, 1956). Van Huis et al. (2013) promoted thet the mass production of insects for food and feed. A few insects such as T. molitor have been commercially mass-produced for a protein source. Heidari-Parsa et al. (2018) showed that yellow mealworm has a high nutritional value and is free of pathogens and larvae of widely reared for animal feed, fish bait, and human consumption (Siemianowska et al., 2013). Temperature is one of the most important abiotic factors that affect the physiology, reproduction and their geographic distribution of yellow mealworm (Bonato, 1992; Engelmann, 1998; Gordon, 1998; Keena, 2006; Sanchez Ramos et al., 2007; Chidawanyika, 2010). Kontodimas et al. (2004) and Wang et al. (2004) had used linear and nonlinear models to determine the relationship between temperature and developmental rates of most insects. Logan et al. (2006) showed that the metabolism of cold-blooded organisms is strongly affected by temperature, and with an increase of temperature in a certain range metabolism and growth become fast .with increasing temperature upper this range, the growth rate slows down or stops, eventually leading to death (Young and Young, 1998). Some research calculated the effect of temperature on some biological features of *T. molitor* (Altman and Dittmer, 1973; Kim et al., 2015; Koo et al., 2013; Ludwig, 1956; Martin et al., 1976; Mutchmor and Richards, 1961; Punzo, 1975; Punzo and Mutchmor, 1980; Spencer and Spencer, 2006). In this study, thermal models were used to the simulation of the relationship between temperature and development and an estimate of the lower, optimum, and upper temperature of T. molitor and select the appropriate model with a view to goodness-of-fit.

2 Materials and Methods

2.1 Experiment conditions

The experiment was performed at the five constant temperatures including 20, 23, 25, 27, 30(±1), and RH and a photoperiod of 12:12, 65±5%RH). for this reason the fifteen males and females were randomly selected from the colony and put in a dish to mating and reproduction. Thirty containers and one egg were laid per container and larva feed by bran and carrots. At each temperature, 30 samples (*T. molitor*) were placed. The larval stages were carefully recorded daily and continued until their death. The survival rate at each temperature was calculated. All stages of life were determined by growth rates.

2.2 Developmental rate models

The performance of 2 linear and 12 nonlinear regression models that describe the effect of temperature on the development of insects were compared (Table 1). Evaluation of each model was based on the following criteria: Statistical parameters to evaluate the accuracy of each model that include the coefficient of determination, or R^2 and residual sum of squares or RSS. Both parameters provide the necessary information to determine the goodness of fit and usefulness of each model to simulate experimental data. Higher R^2 values and lower RSS values for models with more parameters do not confirm their higher accuracy, so other methods are needed.

The Akaike information criterion is expressed as AIC (Akaike, 1974). AIC is an index independent of the number of parameters and Corrected Akaike Information Criterion (AICc) is calculated by the following equation (Hurvich and Tsai, 1993).

$$AICc=n\ln\left(\frac{SSE}{n}\right)+2P+\frac{2P(P+1)}{n-P-1}$$

In this equation, P is the number of parameters in the model, n is the number of observations, and SSE the sum of squares of error (remaining). The smaller the AIC or AICc, indicates that the higher the accuracy of the model (Akaike, 1974).

Models that provide a better estimate of the biological parameters of T_0, T_{max}, and T_{opt} And their predicted have closer values to the experimental results, is more valuable for simulating the growth and development of the insect at different temperatures.

2.3 Stastical analysis

The developmental stages of the experiments were obtained at different temperatures.Comparison of the growth period of different stages of insect stages by analysis of variance and comparison of means with turkey test at 5% level based on the completely randomized design with SPSS software. A normality test was performed before data were analyzed. Analysis of all models in ArthroThermoModel software (in abbreviation ATM) is derived from three words, Arthropod, Thermal, and Models. This software is designed on MATLAB software mentioned by (Mirhosseini et al., 2017).

3 Results

3.1 Development

The developmental time of T.molitor and the duration of the various growth stages are shown in the (Table 2). The period of development in all stages of life (eggs, larvae, pupae, and full-grown insects) has been significantly affected by different temperatures (20, 23, 25, 27, and 30°C) (P-value = 0.000 df=4; F=0.91).The result showed that *T. molitor* was able to complete its life cycle at all temperatures from 20 to 30°C, but at 30°C growth was not increasing. The development time of T.molitorvaried from 203.70 days at 20°C to 140.18 days at 30°C. As the temperature increased from 20 to 27°C, the development time was reduced. The increase in temperature from 27-30°C increased the development time from 118.28 days to 140/18 days and caused a deviation from the linear model. At the temperature of 27°C, in which the time required to complete the total period of the life cycle was calculated 142.1 ± 1.41 days. The length of the first larval period was greater than the second, third, and fourth instar. And at the temperature of 27°C period of larve2 was more than other instars. At the 27°C temperature, the total time required to complete the immature period was obtained on 111.28 d. At 30°C, mortality was higher in the embryonic stage, while in other temperatures the mortality of larvae and pupae was estimated to be higher. Increasing the temperature to 27°C linearly increase and then the slowdown was observed.

Table 1 Mathematical models for describing the effect of temperature on the development of *T. molitor* L.

Equation	Model	(Table1)
R=a+bT $T_0 = -\frac{a}{b}$ $k=\frac{1}{b}$	Ordinary Linear Model or DD Model	Cambell et al. (1974)
$DT=k+T_0D$	Ikemoto Linear Model	Ikemoto and Takai, 2000) (Table1)
$R(T)=R_m \times \exp\left[\frac{-1}{2}\left(\frac{T-T_m}{T_\sigma}\right)^2\right]$	The Pradhan –Taylor Model	(Arbab et al., 2016)
$R(T)=\frac{K}{1+e^{a-bT}}$	The Davidson (Logistic) Model	(Davidson, 1942; Davidson, 1944)

$R(T)=\Psi\times\left[e^{\rho\times T}-e^{\left(\rho\times Tmax-\frac{Tmax-T}{\Delta T}\right)}\right]$	Logan-6 Models	(Logan et al. (1976)
	Lactin's Models	Lactin et al. (1995)
$R(T)=e^{\rho\times T}-e^{\left(\rho\times Tmax-\left(\frac{Tmax-T}{\Delta T}\right)\right)}$	Lactin1	
$R(T)=e^{\rho\times T}-e^{\left(\rho\times Tmax-\left(\frac{Tmax-T}{\Delta T}\right)\right)}+\lambda$	Lactin2	
$R(T)=a\times(T-T_0)^n\times(T_{max}-T)^m$	Analytis/Kontodimas model	(Kontodimas et al., 2004
$R(T)=a\times T(T-T_0)\times\sqrt{T_{max}-T}$	Briere-1	Briere et al. (1999)
$R(T)=a\times T(T-T_0)\times\sqrt[m]{T_{max}-T}$	Briere-2	
$D(T)=\frac{2}{D_{min}\left(e^{k(T-T_{opt})}+e^{-\lambda(T-T_{opt})}\right)}$	ModifedJanisch's Models	(Janisch, 1932, Analytis, 1981, Kontodimas et al., 2004).
$R(T)=a_0T^3+a_1T^2+a_2T+a_3$	Polynomial 3rd order	Harcourt and Yee (1982)
$R(T)=c\left(1-e^{-k(T-T_0)}\right)\left(1-e^{k_2(T-T_u)}\right)$	Performance Models	Shi et al. (2011)
$R(T)=m(T-T_0)\left(1-e^{k_2(T-T_u)}\right)$		
$R(T)=r_m\left[\frac{T_u-T}{T_u-T_{opt}}\right]\left[\frac{T-T_0}{T_{opt}-T_0}\right]^{(T_{opt}-T_0)/T_u-T_{opt}}$	Beta Model	Yin et al. (1995)

Data showed that here was a significant difference between the lifespan of adult insects at different temperatures. Adult insects survived at temperatures of 20, 23, 25, 27, and 30°C on 23.37, 26.04, 29.17, 31.62, and 27.41, respectively. The highest and shortest lifespans of adult insects were at temperatures of 27 and 20°C, respectively. These results showed that with increasing temperature from 20 to 27°C, more adults survived. At the temperature of 27°C, adult insects survive more.Insects had the longest life cycle, the longest embryonic period, larvae, pupae, and immature stages, and the shortest lifespan of adult insects at 20°C, while the shortest life cycle, the shortest embryonic, larval, pupal, immature period and total life cycle period were obtained at 27°C. The whole period of life and the embryonic and larval and pupal periods at temperatures of 25 and 30°C did not differ significantly.

Table 2 *T. molitor* development time and duration of life stages at different temperatures.

Stage(°C)	**30**	**27**	**25**	**23**	**20**	**V_{alue}**

Egg	7.25±0.23 [c*]	6.82±0.25[c]	08.01±0.27[c]	10.32±0.28[b]	12.55±0.43[a]	0.000
Larve$_1$	28.96±0.44[c]	23.17±0.39[d]	31.58±0.61[c]	37.46±1.05[b]	43.18±0.84[a]	0.000
Larve$_2$	29.18±0.32	23.27±0.34[d]	30.10±0.45[c]	35.71±0.92[b]	41.92±0.80[a]	0.000
Larve$_3$	29.74±0.43[c]	23.37±0.42[d]	29.20±0.60[c]	35.03±0.96[ba]	42.59±0.77	0.000
Larve$_4$	30.03±0.38[c]	23.10±0.52[d]	29.06±0.48[c]	34.10±0.70[b]	41.55±0.91[a]	0.000
Pupa	15.00±0.40	12.55±0.31[d]	15.10±0.47[c]	18.07±0.50[b]	21.88±0.43[a]	0.000
Development Time	140.18±1.15[e]	111.28±0.81[d]	143.07±2.28[c]	170.71±3.51[b]	203.70±2.65[a]	0.000
Adult	27.41±0.94[b]	31.62±1.21	29.17±0.94[ab]	26.04±0.80[bc]	23.37±0.94	0.000
Life span	167.59±1.53[c]	142.93±0.41[d]	172.24±2.55[c]	196.75±3.82[b]	227.07±2.90[a]	0.000

*The similar letters in the rows indicate a significant difference between the data at different temperatures.

3.2 Survival rate

Data showed that the highest survival was observed at 25 and 27°C (96%). The mortality in young larvae was higher and then in the embryonic and pupal stages (Table 3). At the temperature of 30°C, all mortality occurred in the embryonic stage, while mortality was observed in the embryonic stages and larvae and pupae of the insects tested at 20°C.

Table 3 The survival rate of T.molitor at different temperatures.

Survival rate (%)	**Temperature (°C)**
90.00	20
93.3	23
96.6	25
96.6	27
90.01	30

3.3 Model evaluation

In the linear regression model the lower temperature threshold (T_0) was 11.9325°C and the required degree-days (k) for development were calculated 1767.7425 DD. According to the Ikemoto and Takai model, this amount was 12.0658°C and 1748.909 DD respectively. The linear regression model was also calculated by removing the temperature of 27°C .The relationship between developmental rates and the temperature was assessed only by ordinary and Ikemoto and Takai linear regression models.the lower temperature threshold (T_0) in this research can be estimated with ordinary linear model, Ikemoto linear model, Briere-1, 2, Analytis-3/Kontodimas, Ratkowsky model and Beta model about 11.9325, 12.0658, 9.889, 9, 9.503, 1.318 and-18.22. According to experimental information and R_2 values ordinary linear model have the best estimate of T_0, The upper threshold of temperature (T_u) obtained by Logan-6, Lactin-1, 2, Briere-1, 2, Analytis-3/Kontodimas, Janisch/Rochat, Ratkowsky mode, Beta model about 35.21, 36.79, 43.78, 38.91, 41, 41.23, 37.36, 43.03, 44.24 and 32.9 respectively Due to the minimum amount of AICc, the model of Lactin-1 and Janisch/Rochat have the best estimated for T_u. about 36.79 and 37.36°C. The highest amount of R^2 and the lowest for AICc were estimated by Janisch/Rochat model.The optimum temperature for development was estimated with models of Pradhan-Taylor, Janisch/Kontodimas, and beta model about 32.44, 20.35, and 28.31 it seems that beta model hasthe best estimate of the optimum temperature based on experimental data. In addition among the models may estimate T_u and $T_{0,}$ the Briere1 have the best goodness of fit 9.889 (T_0) and 38.91 (T_u).

Table 4 Evaluation of various models to describe the effect of temp on the development of *T. molitor* L.

Parameters	Parameters	Estimate	RSS	R^2	dfe	R^2_{adj}	RMSE	AICc
Linear regression	a	-0.00675		0.92885		0.89328	-	-
	b	0.00056						
	K	1767.7425						
	T_0	11.9325						
	P-$_{value}$	0.036229						
IkemotoandTakai (without 27°C)	K	1748.9095		0.92855	-	0.89282	-	
	T_0	12.0658						
	P-$_{value}$	0.0072962						
Pradhan-Taylor	R_m	0.009429 (-0.1149, 0.1338)	1.0349e-06	0.8877	1	0.6631	0.0010	-54.67
	Tm=topt	32.44 (-299.4, 364.3)						
	Tσ	10.09 (-183.6, 203.8)						
Davidsons logistic	a	6.935 (-7.704e+04, 7.706e+04)	9.0959e-06	0.0130	1	-1.9609	0.0030	-45.9759
	b	0.6128 (-3898, 3899)						
	k	0.006685 (-0.03574, 0.04911)						
Logan-6	Δ_T	4.895	1.8377e-06	0.8006	0	NaN	NaN	-50.3731
	Ψ	0.0007461						
	ρ	0.1093						
	T_U	35.21						
Lactin-1	Δ_T	5.736 (-54.99, 66.46)	5.5856e-07	0.9394	1	0.8182	7.4737e-04	-57.1368
	ρ	0.1743 (-1.671, 2.02)						
	T_U	36.79 (-244.9, 318.5)						
Lactin-2	Δ	1.187	6.7670e-07	0.9266	0	NaN	NaN	-54.3693
	λ	-1.006						
	ρ	0.00053						
	T_U	43.78						
Briere-1	T_0	9.889 (-206.6, 226.4)	8.3559e-07	0.9093	1	0.7280	9.1410e-04	-55.5257
	T_u	38.91 (-471.9, 549.7)						
	a	5.254e-06 (-0.0001683,						

		0.0001788)						
Briere-2	T_0	9	6.3367e-07	0.9312	0	NaN	NaN	-54.6321
	T_u	41						
	a	9.259e-06						
	n	4						
Analytis-3/Kontodimas	T_0	9.503 (-136.6, 155.7)	1.1111e-06	0.8794	1	0.6383	0.0011	-54.3859
	T_u	41.23 (-395.1, 477.6)						
	a	1.892e-06 (-8.538e-05, 8.917e-05)						
Janisch/Kontodimas	D_{min}	176.8	2.8887e-06	0.6866	0	NaN	NaN	-48.564
	K	0.02565						
	λ	0.2211						
	T_{op}	20.35						
Janisch/Rochat	c	0.02267	2.7826e-07	0.9698	0	NaN	NaN	-57.924
	T_u	37.36						
	a	0.9174						
	b	1.102						
Polynomial	a_0	8.264e-06	3.5492e-31	1	0	NaN	NaN	-277.9989
	a_1	-0.000512						
	a_2	0.01085						
	a_3	-0.07348						
Performance-2	K_2	0.8511	6.6982e-07	0.9273	0	NaN	NaN	-54.4102
	T_0	11.42						
	T_U	43.03						
	m	0.000543						
Ratkowsky mode	k	0.6974	1.7558e-05	0.9477	0	NaN	NaN	-41.3451
	T_0	1.318						
	T_u	44.24						
	c	0.003619						
Beta model	T_0	-18.22	2.1689e-06	0.7647	0	NaN	NaN	-49.7104
	T_U	32.9						
	T_{opt}	28.31						
	r_m	0.00932						

4 Discussion

According to Johnson (2010), the length of the embryonic period, larvae, pupae, and adult insects were 7-14 days, 1-3 months, 10-20, and 30 days, respectively, and the optimum temperature for *T. molitor* growth and development was 27°C. The results obtained in the present study also confirm the mentioned courses. The researchers found that the life cycle of *T. molitor* lasts about 280 to 630 days. The larval period is about 3 to 4 months at ambient temperature, but up to 18 months can complete its period depending on ambient temperature (Makkar et al., 2014).

Lowest and highest values of duration of lifespan in the Spencer and Spencer (2006) research was 75-90d and in Urs and Hopkins (1973) study was varied from 181, 189 and 196. According to Hill (2002) and

Hardouin and Mahoux (2003) studies minimum and maximum amount was 90 and 120. In this research about 7-12 d take to hatch eggs in temperature between 20-30 while it was 4 d at 26–30°C in the study of Cotton (1927) and Siemianowska et al. (2013) and was about 34 d to hatch at 15°C (Kim et al., 2015). Ribeiro et al (2018) showed that the temperature on development and metabolic rate in the *T. molitor* development rates were quantified at temperatures ranging from 15.2 to 38.0°C and *T. molitor* growth was affected by temperature, with an optimum at 31°C and it was reduced at 37°C. Mellanby (1932) found that *T. molitor* would die after 24h exposure to 38°C and Punzo and Mutchmor (1980) also mentioned that the *T. molitor* survival was 100 % at 25°C even at very high and low relative humidity, while survival was reduced at 10 and 35°C. The result of Mc Clements et al. (2003) and Marcato et al. (2008) study are in the line of the the curret study the young larvae had the highest mortality and according to the some other researshers finding ,the temperature that required to mass breeding this insect is 25–28°C (Kim et al., 2015; Koo et al., 2013; Ludwig 1956; Punzo, 1975; Punzo and Mutchmor, 1980; Spencer and Spencer, 2006).

The lower temperature threshold in the present study was about 12°C based on linear models and about nine based on several nonlinear models, which is almost close to previous researchso thatshow the minimum temperature required for the growth adults of mealworms was 10°C and several nonlinear models calculated a high-temperature threshold of about 36 to 38 and some models calculated a range of 41-44 before. Additionally, the previous researches have shown that the high temperature threshold is about 35°C (Martin et al., 1976; Punzo and Mutchmor, 1980). Data showed that the closest estimate for the high-temperature threshold is the Lactin 1 nonlinear model and the maximum temperature is about 35°C (Martin et al., 1976; Punzo and Mutchmor, 1980). Overally in the current study, the beta model had the best and closest estimate, which is about 28°C and it was differ with the previous studies (Murray, 1960, 1968; Punzo, 1975).

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