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Article *in* International Journal of Tropical Insect Science · January 2022

DOI: 10.1007/s42690-021-00707-0

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ORIGINAL RESEARCH ARTICLE

Effect of population density and photoperiod on larval growth and reproduction of *Tenebrio molitor* (Coleoptera: Tenebrionidae)

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Received: 22 November 2020 / Accepted: 24 November 2021

Abstract

The mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) is the most consumed insect in the world. This study aims to optimize the space in the mass-rearing facilities of this insect, as well as to evaluate the effect of photoperiod on the reproduction and growth of the larvae. The two cross-over factors studied were photoperiods regimes (8L:16D and 0L:24D) and four density treatments (0.25; 0.75; 1; 1.5 ind/cm^2) under rearing conditions of 28 ± 1 °C and 70 ± 5%relative humidity. Statistical analysis showed that density and photoperiod ($P > 0.05$) had no effect on pupal development. Moreover, population density and photoperiod had no effect on pupal development and adult survival. However, average fertility was affected by population density and the optimal population density was 0.25 adults/cm^2.Photoperiod had no effect on the fertility of *T. molitor*. Regarding total fecundity, there is no interaction between the two factors. The fecundity of *T. molitor* was significantly affected by population density. The highest fecundity was obtained at 1.5 adult/cm^2. The optimal combination of photoperiod and density for larval development was of 0.25 ind/cm^2 with 8 h of light per day. The conversion rate was not affected by the factors studied. These findings enable to optimize the mass-rearing of *T. molitor* and reduce the cost and environmental impact of these facilities.

Keywords Mass-rearing · Edible insects · Yellow mealworm · Insect farming

Introduction

Since the world population is continuously increasing, the demand for protein alternatives for food and feed is rising (Tilman and Clark 2014). As a result, the world is under immense pressure to satisfy human nutritional needs (Stehfest et al. 2009). Animal farming pastures and animal feed crops account for more than two thirds of all arable lands (Steinfeld et al. 2006). Meat and other proteins from livestock, particularly cattle, are inefficient and require unsustainable costs and activities that are harmful to the environment (De Vries and de Boer 2010). Insect-based sources of proteins and foods have been proposed to reduce the detrimental effect of meat processing activities on the ecosystem while satisfying population demand (Wegier et al. 2018). Insects show high levels of bioconversion of agricultural and food waste residues (Sun-Waterhouse et al. 2016) and therefore represent ideal sources of proteins to help improve protein conversion efficiency (van Huis 2017).

There are more than 2000 edible species of insects worldwide, however, only a few are commercially (Megido et al. 2014; Sogari et al. 2019; Bordiean et al. 2020). produced (Jongema 2015). Among them, the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) is the most consumed insect in the world. The larvae of *T. molitor* are already used as pet food, providing promising alternative for animal feed (Finke 2002; van Huis 2013). Not only are mealworms suitable as animal feed, but also often considered suitable for human feed (Li et al. 2013, 2015). Mealworms have a higher nutritional value than beef and chicken since they contain all essential amino acids (Rumpold and

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Published online: 13 January 2022

Schlüter 2013). The yellow mealworm is already one of the most commonly industrially reared insect species in Europe Mealworms are inexpensive, easy to rear and show much less harmful effects on the environment compared to farm animals (Wang and Zhang 2015). Many substrates from the agricultural, baking, and brewing industries can be converted by this species into biomass (Oonincx et al. 2015; van Broekhoven et al. 2015). This insect has undergone extensive research in order to validate its nutritious importance and susceptibility to toxic compounds (e.g. mycotoxins, pesticides, heavy metals) (Bordiean et al. 2020). Yellow mealworms will most likely be used as food on a wide scale in the near future, either as a whole or just for certain compounds (chitin, fatty acids, amino acids, proteins) (Bordiean et al. 2020).

One of the major challenges for the insect-producing industry in terms of achieving reliable, cost-effective, and sustainable insect production for food and feed has been the development of diets capable of sustaining and optimizing insect growth and development (Jensen et al. 2017; Heckmann et al. 2018).

The economic production parameters of mealworms include reduced space need, commercial production capability, high conversion efficiency and relative use of organic waste as a food source (Ramos-Elorduy et al. 2002).

Adult density is a major factor impacting reproductive success that can be easily manipulated to optimize egg production in a mass rearing facility (Morales-Ramos et al. 2012). Similarly, photoperiod is known to play an important role affecting larval weight and larval development time, however this factor has been poorly investigated in *T. molitor*. The aim of this research is to assess the impact of both larval density and photoperiod on the reproduction and larval growth of *T. molitor* to optimize the rearing system capabilities. Increasing *T. molitor* output efficiency will benefit the different applications that are using the insect.

Materials and methods

Colony maintenance

The study was conducted in the entomology laboratory at the *Hassan II Agronomic and Veterinary Institute* (Agadir, Morocco), with the following geographical coordinates: 30°21′12.0 "N 9°28′38.8 "W).

The initial *T. molitor* colony was obtained from the "International Foundation for Wildlife Conservation" then mass-reared at the IAV-CHA entomology laboratory. To avoid heterogeneity in adult ages, nymphs (1–2 days old) were collected from the initial stock. These homogeneous nymphs were introduced in cylindrical plastic boxes (diameter = 10 and height = 12 cm) containing 70 g of wheat bran and 30 g of crushed dairy cows feed (15% of crude protein content) manufactured by (ALF ISSEN company).

The boxes were amended with a piece of fresh potato to provide moisture each 4 days. The rearing chamber conditions were maintained at a temperature of 28 ± 1 °C, 70 ± 5% RH and at optimal ventilation. After 5 ± 2 days, emerged adults were counted in each box. The eggs were collected every 10 days using a sieve that separates the adults from the substrate containing the eggs and incubated at the same conditions. The adults were returned to their relative boxes with new substrate at the same proportions for the next egg-laying generation. After 40 days, the number and weight of the larvae as well as the weight of the unused substrate were calculated.

The rearing chambers were separated by an opaque fabric to ensure two different photoperiod regimes 8L:16D (P1) and 0L:24D (P2) with 16 replicates per regime. For each photoperiod regimes, four insect densities were applied (D1 = 0.25; D2 = 0.75; D3 = 1 and D4 = 1.5 ind/cm^2), which respectively correspond to 20; 40; 80 and 120 individuals per box (four replicates per density).

Assessment of colony growth

The larvae were weighed in a weighing balance (SBA 32, 0.1 mg, by SCALTEC). The experimental design adopted was the completely randomized block.

After 40 days, larval number, larval weight and the weight of remaining substrate were assessed following the experimental zootechnical parameters:

$$Weight\ gain = (Final\ weight - Initial\ weight)$$

$$Conversion\ rate = \frac{weight\ gain}{the\ initial\ weight\ of\ the\ feed - the\ final\ weight} \times 100$$

In addition to these zoological parameters, the duration of pupation stage, emergence rate, total fecundity and average fertility (the number of eggs hatched per adult) were monitored.

$$Average\ fertility = \frac{total\ fecundity}{the\ number\ of\ adult\ females\ per\ box}$$

The calculation of the number of larvae and their weighing, as well as the weighing of the remaining substrate, was made according to the same protocol every 10 days; the operation begins at 9:00 am over a period of 5–7 h and with the help of 3 students. In order not to influence the results, we first proceed to the separation of all the larvae present in the boxes of the substrate.

As for the collection of the eggs, the operation was done every 10 days. First the new substrates used for oviposition

were prepared, then adults were separate at around 4:00 pm using a sieve. This total operation did not exceed 2 h.

The duration of the pupal stage (from start of the trial until adult emergence minus one day), is the average age of the test nymphs in adults.

To determine the final emergence rate in each box, the following equation is used:

$$Emergence\ rate = \frac{Number\ of\ emerged\ adults\ per\ box}{number\ of\ nymphs\ per\ box} * 100$$

To measure egg fertility, we first harvested the eggs along with their substrate every 10 days and incubated them in the rearing room for 40 days (the time for the larvae to hatch and be visible for calculation). The sum of these harvests is added to calculate the number of larvae produced per adult during the cycle.

The conversion rate is the ability of the insect to convert feed into larval biomass. A simple equation was used to calculate it: the weight accumulated by the larvae divided by the weight of substrate consumed.

Data analysis

The statistical analyses were performed using R software (R Development Core Team 2021). Data were analyzed using ANOVA and generalized linear model (GLM, R function glm) with a Gaussian error distribution. Post hoc pairwise comparisons were made using Tukey's HSD test (R function glht from the R package multcomp; (Hothorn et al. 2008)).

Results

Adult mortality rate

The mortality rate ranged from 12 to 25%. The different treatments had no effect on mortality rate (GLM, $\chi^2_{7,24}$ = 507.63, $p = 0.056$; Fig. 1).

Total fecundity

Statistical analysis showed no interaction effect between density and photoperiod on total fecundity (GLM, $\chi^2_{3,24}$ = 1326.48, $p = 0.11$; Fig. 2). There was no significant difference between the photoperiod regimes (GLM, $\chi^2_{1,29}$=0.49, $p = 0.21$) on the reproduction of *T. molitor*. However, a strong difference was observed between densities (GLM, $\chi^2_{3,27}$ = 30.29, $p < 0.001$; Fig. 2). The highest the densities generated the highest number of hatched larvae.

Average fecundity

Our analysis showed no interaction effect between density and photoperiod on the number of eggs laid per female (GLM, $\chi^2_{3,24} = 382$, $p = 0.34$; Fig. 3) and no significant difference was observed between the photoperiods (GLM, $\chi^2_{1,29}$ = 121, $p = 0.3$; Fig. 3). However, there is a very highly significant effect of density regimes on average fertility rate (GLM, $\chi^2_{3,26} = 35{,}110$, $p < 0.001$; Fig. 3). The lower the density and the higher was the reproduction rate.

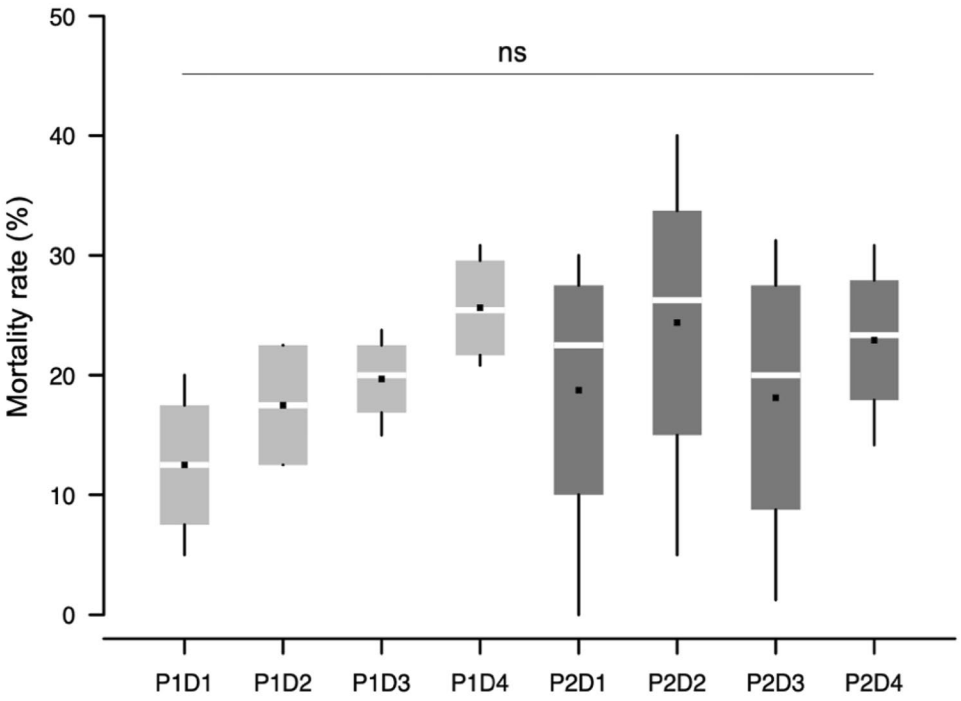

Fig. 1 Mortality rate of *Tenebrio molitor* under different conditions of photoperiods (P1 = 8L:16D; P2 = 0L:24D) and densities (D1 = 0.25; D2 = 0.75; D3 = 1 and D4 = 1.5 ind/cm2). Box plots show the mean (black square), median (white line), and 25–75% percentiles. Whiskers show all data. No statistically significant differences were detected among treatments (GLM, Tukey's HSD test, $p > 0.05$)

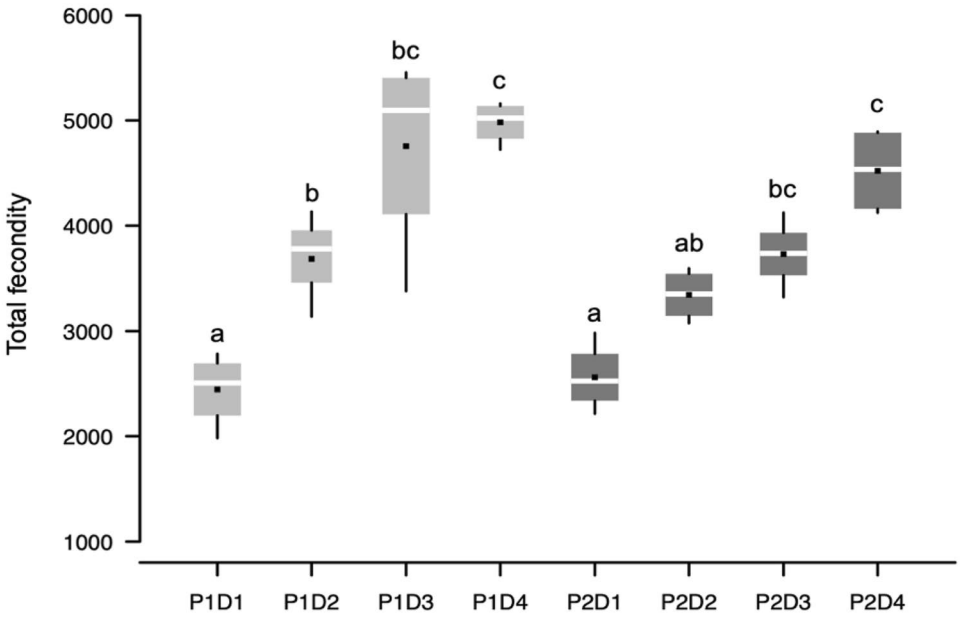

Fig. 2 Total number of eggs laid by *Tenebrio molitor* under different conditions of photoperiods (P1 = 8L:16D; P2 = 0L:24D) and densities (D1 = 0.25; D2 = 0.75; D3 = 1 and D4 = 1.5 ind/cm2). Box plots show the mean (black square), median (white line), and 25–75% percentiles. Whiskers show all data. Letters indicate significant differences between treatments (GLM, Tukey's HSD test, $p < 0.05$)

Larval weight

Our data showed no significant interaction between density and photoperiod on larval weight of larvae (GLM, $\chi^2_{3,24} = 768.32$, $p = 0.07$; Fig. 4). There was no effect of photoperiod on larval weight (GLM, $\chi^2_{1,29} = 208.42$, $p = 0.16$; Fig. 4), however we observed an effect of density on larval weight (GLM, $\chi^2_{3,24} = 1107.64$, $p = 0.017$; Fig. 4). Under photoperiod P1 (8L:16D), the larvae weight was higher when the density was of 1.5 ind/cm2 than when the density was of 0.25 ind/cm2 (Tukey's HSD test, $p = 0.024$; Fig. 4).

Conversion rate

The statistical analysis showed no interaction effect between photoperiod and density (GLM, $\chi^2_{3,24} = 0.009$, $p = 0.056$; Fig. 5) on conversion rates. We also found no significant difference between densities (GLM, $\chi^2_{3,24} = 0.001$, $p = 0.091$;

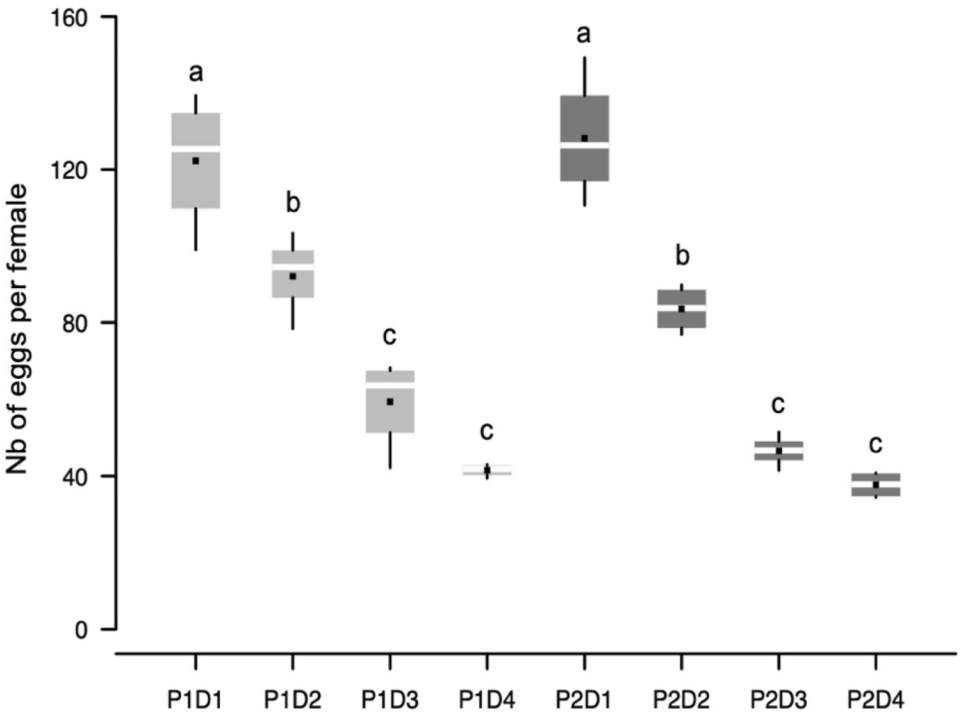

Fig. 3 Mean number of eggs laid per adult female of *Tenebrio molitor* under different conditions of photoperiods (P1 = 8L:16D; P2 = 0L:24D) and densities (D1 = 0.25; D2 = 0.75; D3 = 1 and D4 = 1.5 ind/cm2). Box plots show the mean (black square), median (white line), and 25–75% percentiles. Whiskers show all data. Letters indicate significant differences between treatments (GLM, Tukey's HSD test, $p < 0.05$)

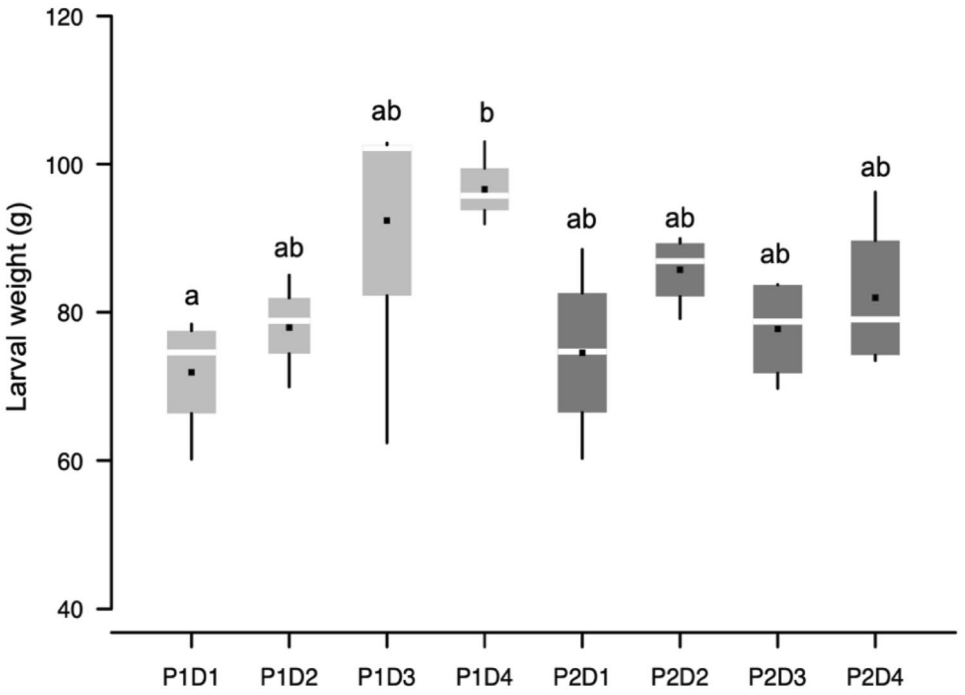

Fig. 4 Weight of larvae of *Tenebrio molitor* after 30 days, under different conditions of photoperiods (P1 = 8L:16D; P2 = 0L:24D) and densities (D1 = 0.25; D2 = 0.75; D3 = 1 and D4 = 1.5 ind/cm2). Box plots show the mean (black square), median (white line), and 25–75% percentiles. Whiskers show all data. Letters indicate significant differences between treatments (GLM, Tukey's HSD test, $p < 0.05$)

Fig. 5) nor between photoperiods (GLM, $\chi^2_{3,24} = 0.0001$, $p = 0.68$; Fig. 5) on conversion rate.

Discussion

The results indicate that increasing population density is negatively correlated with fertility in mealworms. The high density inhibits reproduction or egg hatching rates. This inhibition could be explained by cannibalism associated with over population as shown in closely related beetles such as the red flour beetle *Tribolium castaneum* (Via 1999) and confused flour beetke *Tribolium confusum* (Shostak 2014). (Morales-Ramos et al. 2012)reported that the optimal density for mass-rearing was 0.08 ind/cm^2, three times less than the lowest density we used (0.25 ind/cm^2). Moreover, the same study showed that fecundity was

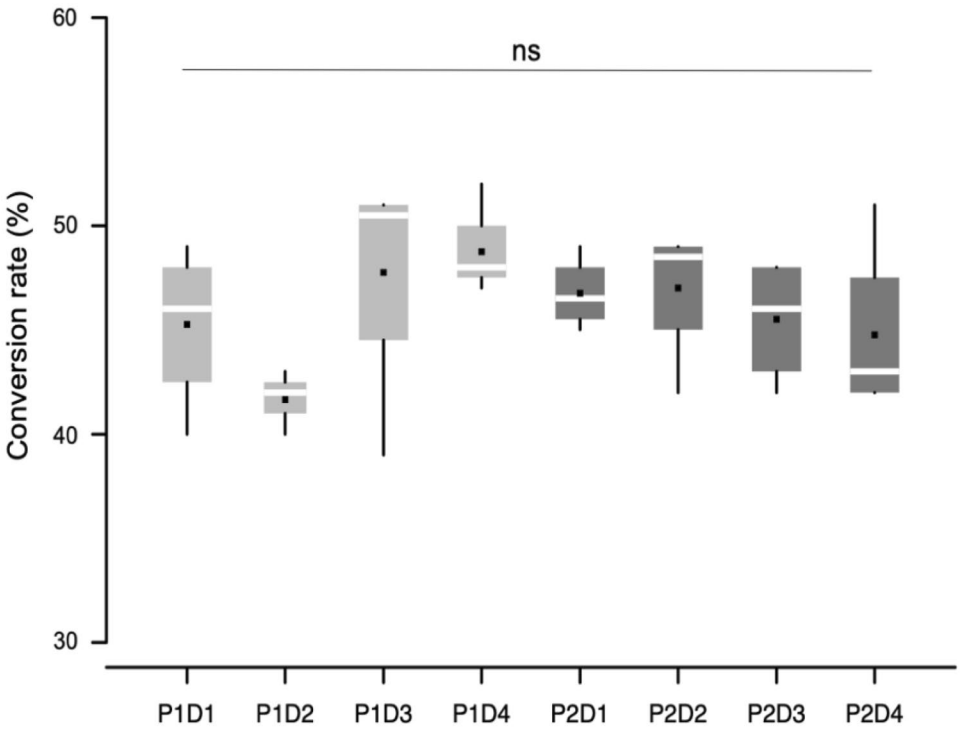

Fig. 5 Conversion rates of *Tenebrio molitor* under different conditions of photoperiods (P1 = 8L:16D; P2 = 0L:24D) and densities (D1 = 0.25; D2 = 0.75; D3 = 1 and D4 = 1.5 ind/cm2). Box plots show the mean (black square), median (white line), and 25–75% percentiles. Whiskers show all data. No statistically significant differences were detected among treatments (GLM, Tukey's HSD test, $p > 0.05$)

reduced under overcrowding conditions. The growth rate of larvae is lower when density increases, which is due to a reduction of food available. Low growth rate can also be a consequence of reduced feed conversion efficiency (Weaver and McFarlane 1990). Our data also show that increased density has a negative effect on adult fertility. Our study contrasts with most studies usually showing an opposite trend (Morales-Ramos et al. 2012; Berggreen et al. 2018; Deruytter et al. 2019). However, the pattern we observed was identified in other beetle species such as the bark beetle *Ips typographus* (Anderbrant 1990) and the red flour beetle *Tribolium castaneum* (Halliday et al. 2015). The overall colony productivity however tends to increase with increasing larval density as the highest biomass produced was reached at the highest larval density. Although overcrowding has a negative effect on the development of *T. molitor* (Peichun and Runjie 2001) it was compensated by a higher number of larvae per box.

Despite these negative effects, we found no difference in conversion rate regardless of photoperiod and density. The photoperiod did not show any significant effect on reproduction, neither on the conversion rate, which means that this parameter does not affect the fertility of the insect. Although interaction effect between photoperiod and larval density was not statistically significant, our data show that under light condition (photoperiod 1, 8L:16D), final larval weight increases with density, which is not the case under full dark condition (photoperiod 2, 0L:24D). These data are in accordance with other studies showing that larval development is influencing by photoperiod (Tyshchenko and Ba 1986) and is optimal under conditions of long days and low in darkness (Kim et al. 2015). Conversely, in natural conditions mealworms were observed to prefer dark and their activity is more important during night than day. This observation can be explained by the behavior of mealworms as they usually cover themselves or dig deep in the rearing substrate to create darkness (Cloudsley-Thompson 1953).

Conclusion

Density is an important parameter to consider in the mass rearing of *T. molitor* as increased density can have a negative impact on its reproduction and growth. To optimize reproduction, a density of 0.25 adults/cm^2 is recommended. On the other hand, if the adults are abundant then a density of 1.5 adults/cm^2 is preferable to have more progeny. The photoperiod does not seem to play an major role in adult reproduction, however further studies are need using a larger spectrum of photoperiodic regimes to fully understand the impact of this parameter. It seems however that larvae accumulate weight faster when exposed to light; but the exact duration remains to be investigated.

Funding No funding was received to assist with the preparation of this manuscript.

Declarations

Ethics approval Not applicable.

Conflicts of interest The authors have no competing interests to declare that are relevant to the content of this article.

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