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The Effect of Larval Density on Pupation Rate and Time to Emergence from Pupation in *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) Reared on Two Different Feeds

Yeşim KOÇ

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Research Article

Corresponding Author
Yeşim KOÇ
ykoc@sinop.edu.tr

ORCID of the Authors
Y.K: 0000-0001-7125-2561

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Abstract

In our research, the yellow mealworm, *Tenebrio molitor* L. was used. The effects of two different foods on the pupation rate and time to emergence from pupation of *T. molitor* at different larval densities were investigated. The experiments were carried out under continuous dark laboratory conditions with a temperature of 27±2°C and a relative humidity of 60%±5%. Two different nutrient and four larval density groups were used in the study. The first nutrient composition consisted of dry yeast and wheat germ (150 gr in total, 1/2) The second nutrient composition consisted of whole wheat flour and corn flour (150 gr in total, 1/1). Insects were bred primarily on the tested nutrient media. Experimental sets were prepared at four different larval densities for both foods. The number of larvae in plastic containers was adjusted to 20, 60, 200 and 600. To ensure equality, all larvae were selected from small larvae. (between 50 mg-120 mg). In our study, pupation rates were high, especially in groups consisting of 20 and 60 larvae. The percentage of pupation decreased at 200 larval densities and sharply decreased at 600 larval densities in both diets. Especially the negative effects of the density are more obvious in the second food. The effects on intensity were more pronounced, especially in pupation of larvae, not in terms of pup time. As a result, it would be advantageous for the larvae density not to be above 200 in terms of getting more yield and increasing the reproduction rate from *T. molitor*, which is demanded in large numbers as live feed. In conclusion, for the production of *T. molitor*, which is commonly used as live feed, it is preferable to have a larval density below 200 in order to get a higher yield and better reproduction rates.

Keywords: *Tenebrio molitor*, larval density, pupation rate, time to emergence from pupation

İki Farklı Besinde Larval Yoğunluğun, *Tenebrio molitor* Linnaeus, 1758 (Coleoptera: Tenebrionidae) un Pupalaşma Oranı ve Pupadan Çıkış Süresine Etkisi

Sinop University, Faculty of Education, Department of Mathematics and Science Education, Sinop, Türkiye

Öz

Araştırmamızda un kurdu *Tenebrio molitor* L. kullanılmıştır. İki farklı besinin, farklı larval yoğunluğunda *T. molitor*' un pupalaşma oranı ve pupadan çıkış süresine etkisi araştırılmıştır. Denemeler, sıcaklığı 27±2°C ve nisbi nemi %60±5 olan devamlı karanlık laboratuvar koşullarında yapılmıştır. Araştırmada iki farklı besin tipi kullanılmıştır. 1. besin; buğday rüşeymi ve kuru mayadan oluşurken, 2. besin; tam buğday unu ve

mısır unun (eşit miktarda) dan oluşmuştur. Öncelikle denenen besin ortamlarında böcekler yetiştirilmiştir. Her iki besin için de dört farklı larva yoğunluğunda deney seti hazırlanmıştır. Plastik kaplarda larva sayısı, 20, 60, 200 ve 600 olacak şekilde ayarlanmıştır. Biyolojik testlerde 50 mg-120 mg arası küçük larvalar tercih edilmiştir. Pupalaşma yüzdesi her iki besinde de 200 larva yoğunluğunda azalmış, 600 larva yoğunluğunda ise keskin bir düşüş olmuştur. Özellikle yoğunluğun ortaya çıkardığı negatif etkilerin 2. besinde daha bariz ortaya çıktığı tespit edilmiştir. Pup süreleri bakımından değil, özelikle larvaların pupalaşmasında yoğunluktaki etkiler daha bariz olmuştur. Sonuç olarak, canlı yem olarak çok fazla sayıda talep gören *T. molitör* den daha fazla verim almak, üreme hızını arttırmak bakımından larva yoğunluğunun özellikle 200 ün üzerinde olmaması avanvajlı olacaktır

Anahtar Kelimeler: *Tenebrio molitor,* larval yoğunluk, pupalaşma oranı, pupadan çıkış süresi

Introduction

The yellow mealworm, *Tenebrio molitor* Linnaeus, 1758 is a pest of stored products. It is also a natural feed for poultry and fish due to its high nutritional content. The ability of its larvae to break down polystyrene and plastic parts, being a natural feed source and being recommended for human nutrition has increased the interest in this insect in recent years. [1-5]. Research efforts to reduce the cost of producing *T. molitor* are focused on determining the ideal rearing conditions and diet [6-15]. On the other hand, larval density, in particular, can significantly affect production efficiency. Previous studies have found that in many tenebrionid species, pupation is delayed or inhibited by crowding, including *T. molitor* [16]. Weaver and McFarlane, [17] reported that larval crowding negatively affects growth in *T. molitor* and found that growth rates decreased with increasing larval densities. A different study reported that larval crowding is well-tolerated by *T. molitor* [18]. Larval crowding reduced pupation in *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) [19]. In *Gnatocerus cornutus* (Fabricaus, 1798) (Coleoptera: Tenebrionidae), increased larval density delayed development and increased mortality and cannibalism [20]. Similarly, in *Alphitobius diaperinus* Panzer, 1797 (Coleoptera: Tenebrionidae), high larval densities were associated with prolonged development times and decreased pupal weight [21]. Further research is needed to determine the optimal larval density in the mass production of *T. molitor*. The growing importance of this species, particularly for use as animal feed, requires a more in-depth understanding of the effect of different rearing conditions and larval density on production. Therefore, it is important to determine the optimal cut-off point for larval density.

Material and Methods

Material

In this study, *Tenebrio molitor,* one of the most common stored grain pests in Türkiye, was used. The larvae used for biological tests were obtained from the stock culture at the Science Research Laboratory of Sinop University Faculty of Education.

Method

This study researches the effect of larval density on pupation rate and time to emergence from pupation in *Tenebrio molitor* (Coleoptera: Tenebrionidae) reared on two different feeds. The trials were conducted under laboratory conditions in continuous dark (CD) with a temperature of 27±2°C and relative humidity of 60%±5. Two different nutrient and four larval density groups were used in the study. The first nutrient composition consisted of dry yeast and wheat germ (150 gr in total, 1/2). The second nutrient composition consisted of whole wheat flour and corn flour (150 gr in total, 1/1). The studies first started by rearing the beetle with this nutrient. The beetles were reared in medium size wide plastic containers (30×20×5 cm). Wood dust was added to nutrients to ease movement and the nutrients were renewed every 10 days. The larvae in the old nutrients were sieved and transferred to the new nutrient. Small pieces (2 for each, 4×4×6 cm) were cut from egg boxes which provide convenience for adults to mate and which is also preferred to lay eggs and they were placed in containers. While the plastic containers were covered to prevent other living beings from being transmitted, small holes were opened on the top side to enable the insects to breathe. Potato was used for humidity (3×3 cm). Potatoes were given wrapped in aluminium foil to prevent from contacting with the nutrients which otherwise may lead to the decaying of the potatoes and moistening of the nutrients as well. Potatoes were changed every 3 days for the litter not to get mouldy. Litter for nutrients was adjusted as 4-5 cm high.

Biological Tests

The research was started with the beetle reared for two generations in the nutrient composition studied. Each nutrient composition was prepared in the abovementioned amounts. For each nutrient composition and different larval density groups (20, 60, 200, and 600 larvae), four sample groups were formed with the beetle taken from the population at different times. Experimental sets were prepared at four different larval densities for both foods. The number of larvae in plastic containers was adjusted to 20, 60, 200 and 600. To ensure equality, all larvae were selected from small larvae. (between 50 mg-120 mg). As the pups formed in the vessels, they were taken into separate containers. The number of pupating larvae and dead larvae were recorded. The dishes were checked every day and the pups were taken the day they were first formed and then placed in petri dishes (9 cm x1.5 cm). Petri dishes were observed daily and the time from pups to adults was recorded. The time to emerge from the pupa was determined by calculating the time at each adult emergence. Three repetitions were performed for each intensity on each food and the results were averaged.

Statistical Analysis

In the study, pupation percentages and time to emergence from pupation (in days) were presented as descriptive statistics and compared based on different larval density groups (20, 60, 200, and 600 larvae). The ANOVA test was used for statistical comparison. The groups that were significantly different in the

ANOVA test were compared with the Tukey test. All statistical hypothesis tests were performed using the R-Project software. The margin of error was determined as 5% in hypothesis testing.

Results

Table 1 presents the descriptive statistics for pupation rates (%) based on larval density groups.

Table 1. *Descriptive statistics for pupation rates for Feed 1 and Feed 2.*

Feed	Group	$\bar{X}$	SD	Min	Max
Feed 1	20 larvae	92.2	1.4	90.7	93.3
	60 larvae	95.9	1.2	94.5	96.6
	200 larvae	83.0	1.5	81.8	84.7
	600 larvae	61.4	3.0	58.8	64.6
Feed 2	20 larvae	83.9	4.4	80.5	88.8
	60 larvae	91.9	1.3	90.8	93.4
	200 larvae	71.4	2.4	68.9	73.6
	600 larvae	51.0	3.1	48.9	54.5

$\bar{X}$: Mean, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 2. *Descriptive statistics for time to emergence (days) for Feed 1 and Feed 2.*

Feed	Group	$\bar{X}$	SD	Min	Max
Feed 1	20 larvae	6.6	1.2	5.0	9.0
	60 larvae	6.7	1.3	5.0	9.0
	200 larvae	6.9	1.2	5.0	9.0
	600 larvae	7.2	1.0	6.0	9.0
Feed 2	20 larvae	6.9	1.1	5.0	9.0
	60 larvae	6.7	1.0	5.0	9.0
	200 larvae	6.7	1.2	5.0	9.0
	600 larvae	7.5	1.0	6.0	9.0

$\bar{X}$: Mean, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 2 presents the descriptive statistics for time to emergence (days) based on larval density groups.

Table 3. *ANOVA test results where pupation percentages for Feed 1 is the dependent variable*

	DF	SS	MS	F	*P*
Group	3	2152.6	717.5	202.100	**<0.001**
Error	8	28.4	3.6		

DF: Degree of freedom, SS: Sum of squares, MS: Mean sum of squares

Table 3 presents the ANOVA test results for pupation percentages considering different larval density groups (20, 60, 200, 600 larvae) for Feed 1. According to the ANOVA table, pupation percentages for Feed 1 were significantly different between larval density groups ($F=202.100$, $P<0.05$). Tukey test, a multiple comparison test, was applied to larval density groups that were significantly different.

Table 4. *Pupation percentages of T. molitor for groups of different larval densities with Feed 1*

	MD	**95% CI**		***P***
		Lower limit	**Upper limit**	
20 larvae-200 larvae	9.2	4.2	14.1	**0.002**
600 larvae-200 larvae	-21.6	-26.6	-16.7	**<0.001**
60 larvae-200 larvae	12.9	7.9	17.8	**<0.001**
600 larvae-20 larvae	-30.8	-35.7	-25.9	**<0.001**
60 larvae-20 larvae	3.7	-1.2	8.6	0.153
60 larvae-600 larvae	34.5	29.6	39.4	**<0.001**

MD: Mean difference, CI: Confidence interval of mean difference

For larval density groups that were found to be significantly different (see Table 3), a Tukey test was performed to determine the group from which the difference originated and the results are presented in Table 4. According to the post hoc analysis, with Feed 1, the group with a larval density of 60 had a significantly higher pupation percentage compared with the groups of 200 and 600 larvae. Moreover, with Feed 1, the group with the larval density of 600 had a significantly lower pupation percentage compared with the groups of 20 and 200 larvae. On the other hand, with Feed 1, the group with a larval density of 20 had a significantly higher pupation percentage compared with the group of 200 larvae.

Table 5. *ANOVA test results where pupation percentages for Feed 2 is the dependent variable*

	DF	**SS**	**MS**	**F**	***P***
Group	3	2859.9	953.3	106.600	**<0.001**
Error	8	71.5	8.9		

DF: Degree of freedom, SS: Sum of squares, MS: Mean sum of squares

Table 5 presents the ANOVA test results for pupation percentages based on different larval density groups (20, 60, 200, 600 larvae) for Feed 2. According to the ANOVA table, pupation percentages for Feed 2 were significantly different between larval density groups (F=106.600, $P<0.05$). Tukey test, a multiple comparison test, was determined to be appropriate to compare larval density groups that were significantly different.

Table 6. *Pupation percentages of T. molitor for groups of different larval densities with Feed 2*

	MD	**95% CI**		***P***
		Lower limit	**Upper limit**	
20 larvae-200 larvae	12.4	4.6	20.3	**0.004**
600 larvae-200 larvae	-20.4	-28.3	-12.6	**<0.001**
60 larvae-200 larvae	20.5	12.7	28.3	**<0.001**
600 larvae-20 larvae	-32.9	-40.7	-25.0	**<0.001**
60 larvae-20 larvae	8.1	0.2	15.9	**0.043**
60 larvae-600 larvae	40.9	33.1	48.8	**<0.001**

MD: Mean difference, CI: Confidence interval of mean difference

For larval density groups that were found to be significantly different (see Table 5), a Tukey test was performed to determine the group from which the difference originated and the results are presented in Table 6. According to the post hoc analysis, with Feed 2, the group with the larval density of 600 had a significantly lower pupation percentage compared with the groups of 200, 20, and 60 larvae. Moreover, with Feed 2, the group with the larval density of 60 had a significantly higher pupation percentage compared to groups with 20 and 200 larvae. On the other hand, with Feed 2, the group with the larval density of 20 had a significantly higher pupation percentage compared to the group with 200 larvae.

Table 7. *ANOVA test results where time to emergence (days) for Feed 1 is the dependent variable*

	DF	**SS**	**MS**	**F**	***P***
Group	3	5.5	1.8	1.328	0.269
Error	116	161.1	1.4		

DF: Degree of freedom, SS: Sum of squares, MS: Mean sum of squares

Table 7 presents the ANOVA test results for time to emergence (days) based on different larval density groups (20, 60, 200, 600 larvae) for Feed 1. According to the ANOVA table, time to emergence from pupation (days) with Feed 1 was not statistically different between groups of larval densities (F=1.328, *P*>0.05).

Table 8. *ANOVA test results where time to emergence (days) for Feed 2 is the dependent variable*

	DF	**SS**	**MS**	**F**	***P***
Group	3	15.0	5.0	4.192	**0.007**
Error	116	138.7	1.2		

DF: Degree of freedom, SS: Sum of squares, MS: Mean sum of squares

Table 8 presents the ANOVA test results for time to emergence (days) according to larval density groups (20, 60, 200, 600 larvae) for Feed 2. According to the ANOVA table, time to emergence from pupation (days) for Feed 2 was significantly different considering larval densities (F=4.192, *P*>0.05). Tukey test, a multiple comparison test, was found to be appropriate to compare larval density groups that were significantly different.

Table 9. *Time to emergence from pupation for T. molitor between groups of larval densities with Feed 2*

	MD	**95% CI**		***P***
		Lower limit	**Upper limit**	
20 larvae-200 larvae	0.3	-0.5	1.0	0.781
600 larvae-200 larvae	0.9	0.1	1.6	**0.014**
60 larvae-200 larvae	-0.0	-0.7	0.7	1.000
600 larvae-20 larvae	0.6	-0.1	1.3	0.151
60 larvae-20 larvae	-0.3	-1.0	0.5	0.781
60 larvae-600 larvae	-0.9	-1.6	-0.1	**0.014**

MD: Mean difference, CI: Confidence interval of mean difference

For larval density groups that were found to be significantly different (see Table 8), a Tukey test was performed to determine the group from which the difference originated and the results are presented in Table 9. According to the post hoc analysis, with Feed 2, the time to emergence (in days) was significantly longer for the group with the larval density of 600 compared to groups with 200 and 60 larvae.

Discussion

Several studies on *T. molitor* Linnaeus, 1758 report negative effects with high larval density, in parallel with our results. Producers often resort to cold exposure to increase production yield in this species; on the other hand, it will be favorable to determine the exact effect of larval density on the reproduction of *T. molitor*. According to Tschinkel and Willson [16] the negative impact of larval crowding in certain tenebrionids is due to physical contact between larvae, or in other words, mechanical stimulation. Although *T. molitor* is comparatively insensitive to mechanical disturbances, pupation was significantly delayed by tactile stimulation (disturbing with chains) and vibration at low larval densities. Weaver and McFarlane [17] hypothesized that in the setting of high larval density, the reduced growth in *T. molitor* was due to reduced feeding opportunities caused by intraspecific competition. The same authors reared one-day-old larvae of *Tenebrio molitor* at 30±1°C at 55%±5% RH at densities of 1, 2, 5, 10, and 20 larvae per 455 mL rearing jar. After one month, larvae reared at a density of 20 were significantly larger compared to those reared at a density of two individuals, which were the smallest. Harada and Spence, [22] investigated the effects of rearing density on the duration of nymphal development in two wing-dimorphic water strider species, namely *Gerris buenoi* Kirkaldy and *Gerris pingreensis* Drake and Hottes (Hemiptera: Gerridae). Accordingly, they noted that under higher larval density, the average nymphal period was significantly reduced by approximately 4-5 days for both species. However, they did not find larval density to be significantly associated with per cent survival in either species. Silva et al. [23] conducted a study to develop a mass production method for *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) in laboratory. Nymphs were reared at egg densities of 100, 200, 300, and 400 per petri dish (diameter 9 cm), and adults at densities of 50, 100, 150, and 200 couples per rearing dish (900 mL). The highest survival rate was recorded in the group with an egg density of 100 (89%). They reported that a density of 100 couples per rearing cage was the best density to improve the quality of the progeny. Ito [24] reported that in *O. strigicollis* and *O. laevigatus*, when the initial nymphal density was 100–400, the average rate of individuals reaching the adult stage was 74–87% while noting that the studied species had a high breeding capacity. In another study on *T. molitor*, reproductive output per female decreased with increasing adult density. In this study, progeny per unit area peaked at a density of 14 adults/dm and then sharply declined. Larval growth was also adversely affected by larval density in *Tenebrio molitor* Linn, possibly due to reduced feeding opportunities as a result of increased competition [25]. In our study, the decreased pupation rates recorded with high larval densities may be

due to nutritional deficiency associated with competition. This decrease was particularly prominent in groups with a larval density of up to 600. Pupation rates were higher at relatively lower densities. This is consistent with the study which concluded that *T. molitor* well adapts to high larval densities and can boost immunity in crowded conditions to prevent infection [26]. However, in our study, particularly at a density of 600 larvae, the pupation rate was 61.4% with Feed 1 and 51% with Feed 2 (Table 1). The higher pupation rate achieved with Feed 1 may be because of the compatibility of its composition to the biological requirements of the species. Pupation began to decrease particularly after a density of 60 larvae, and this decrease was even more prominent after passing a larval density of 200. Overall, with Feed 1, time to emergence was not prominently associated with larval density, that is results were similar for all larval density groups. With Feed 2, time to emergence was significantly longer in the group with a larval density of 600 (Table, 2).

Conclusions

In our study, pupation rates were higher especially in groups with densities of 20 and 60 larvae. The negative impact of crowding was particularly prominent with Feed 2. This may be because Feed 1 was more compatible with the biological requirements of the studied species. The effects of crowding were even more prominent on pupation rates compared to time to emergence from pupation. In conclusion, for the production of *T. molitor*, which is commonly used as a live feed, it is preferable to have a larval density below 200 to get a higher yield and better reproduction rates.

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Conflict of Interests -

Authors Contribution The author read and approved the final manuscript.

References

[1] Finke, M. D. (2015). Complete nutrient content of four species of commercially available feeder insects fed enhanced diets during growth. *Zoo Biology, 34,* 554-564. https://doi.org/10.1002/zoo.21246

[2] Ravzanaadii, N., Kim, S., Choi, W. H., Hong, S., Kim, N. J. (2012). Nutritional Value of mealworm, *Tenebrio molitor* as food source. *International Journal of Industrial Entomology, 25*(1),93-98. https://doi.org/10.7852/ijie.2012.25.1.093

[3] Hong, J., Han, T., Kim, Y. Y. (2020). Mealworm (*Tenebrio molitor* larvae) as an alternative protein source for monogastric animal: A review. *Animals. 10*(11), 2068. https://doi.org/10.3390/ani10112068

[4] Tekeli, A. (2014). Hayvan beslemede alternatif protein kaynağı olarak böceklerin kullanımı. *Türk Tarım ve Doğa Bilimleri Dergisi 1*(4), 531-538.

[5] Van Huis, A. V., Itterbeeck, J. V., Klunder, H., Mertens, E., Halloran, A., Muir, G. & Vantomme, P. (2013). Edible insects: Future prospects for food and feed security. FAO Forestry Paper, Wageningen, 171.

[6] Morales-Ramos, J. A., Rojas, M. G., Shapiro-Ilan, D. I. & Tedders, W. L. (2010). Developmental plasticity in *Tenebrio molitor* (Coleoptera: Tenebrionidae): Analysis of instar variation in number and development time under different diets. *Journal of Entomological Science. 45,* 75-90. https://doi.org//10.18474/0749-8004-45.2.75

[7] Morales-Ramos, J. A., Rojas, M. G., Shapiro-Ilan, D. I., Tedders, W. L. & Tedders, A. W. L. (2011). Self-selection of two diet components by *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae and its impact on fitness. *Environmantal. Entomology, 40,* 1285-94. https://doi.org//10.1603/EN10239

[8] Morales-Ramos, J. A., Rojas, M. G., Shapiro-llan, D. I. & Tedders, W. L. (2013). Use of nutrient self-selection as a diet refining tool in *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Journal of Entomological Science, 48*(3),206-221. https://doi.org//10.18474/0749-8004-48.3.206

[9] Morales-Ramos, J. A., Rojas, M. G., Shelby, K. S. & Coudron, T. A. (2016). Nutritional value of pupae versus larvae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) as food for rearing Podisus maculiventris (Heteroptera: Pentatomidae). *Journal of Economic Entomology, 109*(2), 564-571. https://doi.org/10.1093/jee/tov338

[10] Oonincx, D. G. A. B., Van Broekhoven, S., Van Huis, A. & Van Loon, J. J. A. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS One, 10*, e0144601

[11] Rho, M. S., Lee, K. P. (2016). Balanced intake of protein and carbohydrate maximizes lifetime reproductive success in the mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae*). Journal of Insect Physiology, 91-92,* 93-99. https://doi.org//10.1016/j.jinsphys.2016.07.002

[12] Ribeiro, N., Abelho, M. & Costa, R. A. (2018). review of the scientifc literature for optimal conditions for mass rearing *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Journal of Entomological Science, 53,* 434-454. https://doi.org/10.18474/JES17-67.1

[13] Rojas, M. G., Morales-Ramos, J. A. & Riddick, E. W. (2016). Use of *Tenebrio molitor* (Coleoptera: Tenebrionidae) powder to enhance artifcial diet formulations for *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Biological Control, 100,* 70-78. https://doi.org/10.1016/j.biocontrol.2016.05.018

[14] Rumbos, C. I., Adamaki-Sotiraki, C., Gourgouta, M., Karapanagiotidis, I. T., Asimaki, A., Mente, E., & Athanassiou, C. G. (2021). Strain matters: Strain effect on the larval growth and performance of the yellow mealworm, *Tenebrio molitor* L. *Journal of Insects Food Feed, 7*(8), 1195-1205. https://doi.org/10.3920/JIFF2021.0035

[15] Adamkova, A., Mlcek, J., Adamek, M., Borkovcova, M., Bednarova, M., Hlobilova, V. Knížková, I., & Juríková, T. (2020). *Tenebrio molitor* (Coleoptera: Tenebrionidae)-optimization of rearing

conditions to obtain desired nutritional values. *Journal Insect Science, 20*(5), 24; 1-10. https://doi.org/10.1093/jisesa/ieaa100

[16] Tschinkel, W. R. & C. D. Willson. (1971). Inhibition of pupation due to crowding in some tenebrionid beetles. *Journal of Experimantal Zoology, 176*, 137-146.

[17] Weaver, D. K. & J. E. McFarlane. (1990). The effect of larval density on growth and development of *Tenebrio molitor. Journal of Insect Physiology, 36*, 531-536. https://doi.org/10.1016/0022-1910(90)90105-O

[18] Zaelor, J. & S. Kitthawee. (2018). Growth response to population density in larval stage of darkling beetles (Coleoptera; Tenebrionidae) *Tenebrio molitor* and *Zophobas atratus*. *Agriculture and Natural Resources, 52*, 603-606. https://doi.org/10.1016/j.anres.2018.11.004

[19] Via, S. (1999). Cannibalism facilitates the use of a novel environment in the flour beetle, Tribolium castaneum. *Heredity, 82*(3), 267-275.

[20] Savvidou, N., & C. H. Bell. (1994). The effect of larval density, photoperiod and food change on development of *Gnatocerus cornutus* (F.) (Coleoptera: Tenebrionidae). *Journal of Stored Products Research, 30*, 17-21. https://doi.org/10.1016/0022-474X(94)90268-2

[21] Parween, S. & Begum, M. (2001). Effect of larval density on the development of the lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). *International Pest Control ,43*, 205-207.

[22] Harada, T. & Spence, J. R. (2000). Nymphal density and life histories of two water striders (Hemiptera: Gerridae). *The Canadian Entomologist, 132*(03), 353-363.

[23] Sılva, C. C., Laumann, R. A., Blassıolı, M. C., Pareja, M. & Borges, M. (2008). *Euschistus heros* mass rearing technique for the multiplication of *Telenomus podisi. Pesquisa Agropecuaria Brasileira, 43*(5) 575-580. https://doi.org/10.1590/S0100-204X2008000500004

[24] Ito, K. (2007). A simple mass-rearing method for predaceous *Orius* bugs in the laboratory. *Applied Entomology and Zoology, 42*(4), 573-577. https://doi.org/10.1303/aez.2007.573

[25] Morales-Ramos, J. A., & Rojas, M. G. (2015). Effect of larval density on food utilization efficiency of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology, 108*(5), 2259-2267. https://doi.org/10.1093/jee/tov208

[26] Barnes, A. I., & Siva-Jothy, M. T. (2000). Density–dependent prophylaxis in the mealworm beetle Tenebrio molitor L. (Coleoptera: Tenebrionidae): cuticular melanization is an indicator of investment in immunity. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 267*(1439), 177-182. https://doi.org/10.1098/rspb.2000.0984