

## Cannibalistic behavior of the yellow mealworm (*Tenebrio molitor*) reared in the laboratory

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### Introduction

Cannibalism has been recorded in more than 1,300 species so far, so it has been reported in many arthropods and insect orders, including Coleoptera, and in this behavior, beetles and larvae hunt immobile pupae and eggs [Fernandez *et al.*, 2020]. Cannibalistic interactions act as a self-regulatory mechanism, and in some social insects, larvae or beetles feed on conspecific eggs to improve growth rate, vigor, and fecundity, which is called egg cannibalism [Mastrantonio *et al.* 2021; Jiang *et al.* 2022]. Cannibalism behavior in the *Tenebrio molitor* increases during periods of starvation and fruit deprivation [Ichikawa & Kurauchi. 2009; Via, 1999]. The *Tenebrio molitor* has complete metamorphosis (egg, larva, pupa and beetle). Eggs are oval and sticky [Siemianowska, *et al.*, 2013; Ribeiro, 2017; Van Huis, *et al.*, 2013]. The larval stage begins with the hatching of tiny white larvae [Kim, *et al.* 2015]. After completing several molts larvae turn into pupae. The pupae cannot feed and move, and metamorphosis occurs during this period [Van Huis, 2013; Alves, 2016; Patterson, 2016]. Finally, a beetle with a soft skeleton emerges from the pupa [Cotton, 1927]. Larval cannibalism and pupal defense in two species, *Tenebrio molitor* and *Zophobas atratus*, showed that larvae used pupae that had their abdominal region paralyzed as prey [Via, 1999]. The purpose of this study is to investigate the effect of seven diets and the presence and absence of fruit on the selective cannibalistic behavior of adults and larvae regarding eggs and pupae.

## Materials & Methods

The experiments were performed at the Ecology Laboratory of Razi University in Kermanshah. While performing the experiments, the larvae were kept in a plastic chamber set at 65% relative humidity, 28 °C, and a 12 h photoperiod cycle. Then we prepared 21 plastic containers (dimensions: 22 × 28 × 15 cm) with seven types of diets. The experimental treatments consisted of a control diet (W) containing wheat bran (400 gr), Diet A containing barley flour and wheat flour (200 +200 gr), Diet B includes barley flour and wheat flour (100 +300 gr), Diet C includes chickpea flour and wheat flour (200 +200 gr), 5- Diet D chickpea flour and wheat flour (100 +300 gr), Diet E includes corn flour and wheat flour (200 +200 gr), Diet F includes corn flour and wheat flour (100 +300 gr). To supply water and vitamins, carrot pieces with the same amount were used.

To investigate the selective cannibalism of eggs and pupae by beetle and larvae, first, beetle and larvae were randomly selected from the culture medium and ten samples of beetle were transferred to containers and then 15 pupae and 25 eggs were distributed among them [Pires & Serrão 2011]. In the investigation of the selective behavior of eaten beetle and pupae by larvae, 30 larvae have placed in the containers and then 15 pupae and 10 beetle were distributed among them. In both experiments, the cannibal's samples were allowed to feed on the prey for three hours [Via, 1999]. The number of eggs, pupae, and damaged beetle was counted and recorded. To compare the cannibalism rate of beetles and larvae, they were randomly selected and then put into a period of starvation (not receiving fruit) for 12 hours. then, 30 samples of beetles and larvae and 15 pupae were transferred to the container, and the cannibal's samples were allowed to feed on the pupae for three hours. Then the number of damaged pupae was counted and recorded. To compare the rate of cannibalism of beetle to pupae during 4 periods of starvation (12, 24, 36 and 48 hours), 30 beetles and 15 pupae were placed in each container and the beetles were allowed to attack the pupae for three hours. In the following, the proportion of pupae eaten in 4 periods of 12, 24, 36 and 48 hours were counted and recorded. All statistical tests were performed with Excel (version 2016) and SPSS (version 26) for Windows.

## Results & discussion

In the study of selective cannibalism behavior of beetle, the highest and lowest means of eaten eggs and pupae were recorded in the absence and the presence of fruit treatments, respectively. It was also observed that the nutritional factor was effective on the selective cannibalistic behavior of the adult insect, but did not affect the larvae stage. The data analysis showed that there is a significant difference in the level of cannibalism between the treatments that were subjected to a period of starvation and the treatments that received the fruit (Carrot). Therefore, the presence of fruit reduces cannibalism in all treatments. The result shows that beetles have a greater tendency to eat eggs. The searching behavior of beetles in the bed of their breeding environment, which is the place where the eggs are stored, as well as the lack of movement and the thin covering of the eggs can be the reasons for this behavior of the beetle. In the investigation of the selective behavior of larval cannibalism to beetle and pupae, it was

observed that larvae have a greater tendency to eat pupae and this can be due to the soft, moist body and immobility of pupae compared to beetle.

Also, the food quality of the insect diet affects the cannibalism behavior of the larvae. So the larvae that were grown in the treatments containing a higher percentage of pea bran and a lower percentage of corn bran have lower and higher cannibalism power, respectively. The results of the research on the rate of cannibalism of pupae by beetles after 4 periods of starvation show that the insects that were deprived of fruit for more than 12 hours showed a higher rate of cannibalism. Therefore, the deprivation of fruit and periods of starvation should not be more than 12 hours, and to prevent cannibalism, fruit pieces should be used as a source of water and vitamins in insect breeding. The type of diet between the treatments is effective during the starvation period, so in the treatments that consist of 100% wheat bran (W) and 50% pea bran (C) and among the treatments that were under a 12-hour starvation period, the number of pupae was relatively less, and it shows the influence of the quality of insect diet on cannibalism behavior. Also, the interactive effect of the duration of the starvation period and the type of diet affects the rate of cannibalism of the beetle. The increase of cannibalism in eggs and pupae with periods of food deprivation shows that starvation and the absence of fruit stimulate the search for food and cause the occurrence of cannibalism to maintain the survival of the insect.

## **Conclusion**

In this study, for the first time, the cannibalistic behavior of the yellow flour beetle *Tenebrio molitor* was investigated in the laboratory. The results of the mentioned study showed that beetles and larvae attack eggs and pupae in the absence of sufficient food and fruit, which is called cannibalism. The quality of the insect diet can determine the type of prey of the beetle in its cannibalism. The quality of the diet can determine the type of prey in the cannibalistic behavior. The presence of fruit as a source of water and moisture is necessary and it reduces cannibalism in this species.

**Keywords:** *cannibalism, pupae, starvation period, Yellow mealworm.*

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## **Declaration of conflict of interest:**

The authors declare that there is no conflict of interest.

## **Statement on ethics:**

The permit for sampling and working live animals were issued by approval of the Razi University Animal Ethics Committee with the code number of IR.RAZI.REC.1400.018.