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## Comparative study of different artificial diets on the biology of beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae)

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**Abstract.** The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera; Noctuidae), is an important polyphagous insect pest of many vegetable and field crops in most parts of the world. Aimed at studies on insect manage methods including sterile insect technique (SIT), it is necessary to continuously maintain laboratory colonies of insect species on an artificial diet. The current study aimed to evaluate the effects of different protein-based diets (soybean chunks, chickpea, kidney bean, mung bean, pinto bean, white bean, lentil and corn) on the biology and growth indices of *S. exigua*. For this purpose, different artificial diets were prepared and larvae were reared on these diets. The larval survival, larval period, pupation, pupal weight, pupal period, adult emergence, adult longevity, fecundity, fertility and life span were calculated accordingly. The larval survival rates were lowest on lentil, pinto bean and corn diets ( $P < 0.01$ ) and pupation did not occur on these diets. Based on the results, among the other five artificial diets, all the highest studied parameters, the developmental and reproduction values and growth indices, were recorded on soybean chunks and chickpea diets ( $P < 0.01$ ). The larval survival, pupation rates, larval growth index and fitness index have been further optimized when these two protein-based ingredients were mixed together (50:50). Consequently, soybean chunks and chickpea are introduced as the best dry ground protein ingredients in the beet armyworm artificial diet.

**Keywords:** Artificial diet, Beet armyworm, Biological parameters, Growth indices, Pest control

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## Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera; Noctuidae) has a wide host range, occurring as a vital pest of vegetable, field, and flower crops in most parts of the world. Larvae feed on both foliage and fruit, but it is known as a serious defoliator and skeletonize foliage (Capinera, 2017).

The long-term use of insecticides to control the beet armyworm, has led to the development of resistance to many chemical pesticides (Ishtiaq *et al.*, 2012). Therefore, it is important to develop alternative and safe control methods for human health and the environment, such as the sterile insect technique (SIT). SIT is an autocidal method in which the large numbers of the reared insects are sterilized by ionizing irradiation using gamma- or X-rays, and subsequently periodic release of sterile males into the target area leads to suppression of the pest populations especially in F1 generation of lepidoptera. To manage insect pests on an area-wide basis using this technique, we require colonization and mass rearing of the target insect and providing a large number of healthy insects for irradiation (Marec & Vreysen, 2019).

Rearing insect colonies in the laboratory using an artificial diet is more convenient than using a natural medium because it is easier to handle and less prone to microbial contamination. In addition, rearing insects on their natural hosts may not make sense for several reasons, such as seasonal availability, frequent replacement, and variable quality (Abdullah *et al.*, 2000; Nair *et al.*, 2019). Standardized artificial diets have been used in different laboratories

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for mass rearing of *S. exigua*, and in turn, the continuous maintenance of laboratory colonies has become seriously facilitated (Srinivasa Murthy *et al.*, 2006; Lalitha & Ballal, 2015).

A number of techniques for laboratory rearing of *S. exigua* have been described in the literature (Shorey & Hale, 1965; Patana, 1969; Singh & Moore, 1985; Elvira *et al.*, 2010). One of the considerations in preparing an artificial diet is the balanced nutritional content specially macromolecules such as proteins and carbohydrates, which play an important role in the development and reproduction of insects and allow continuous rearing for many generations (Elvira *et al.*, 2010). Several varieties of dry ground beans have been evaluated as the basic ingredient in the *Spodoptera* spp. artificial diets. These include pinto bean (Shorey & Hale, 1965), lima or white bean (Singh & Moore, 1985), mung bean (Abdullah *et al.*, 2000), soy protein or soybean (Srinivasa Murthy *et al.*, 2006; Elvira *et al.*, 2010; Di *et al.*, 2021), lentil (Alfazairy *et al.*, 2012) and chickpea (Nair *et al.*, 2019), and also corn (Pinto *et al.*, 2019). The purpose of this work was finding the best basic ingredient of an artificial diet for large-scale laboratory rearing of *S. exigua*. In this respect, here, optimal larval survival rate, pupation rate, pupal weight, adult emergence, fecundity, fertility, developmental period of different stages, and also growth and fitness indices of *S. exigua* were considered under different protein-based diets.

## Materials and methods

### Insect rearing

Larvae of *S. exigua* were collected from a sugar beet field in Karaj, Iran, and transported to the laboratory along with host plant leaves and kept individually in 50 ml airtight plastic cups on beet leaves until pupation. Adult moths were released into egg-laying boxes which was explained in bioassay procedure. The larvae were reared on an artificial diet which described by Singh & Moore (1985) for three generations, at  $26 \pm 1$  °C,  $30 \pm 5$  % relative humidity and a photoperiod of 16:8 (L:D) h.

### Artificial diet preparation

Larvae were reared on an artificial diet modified from a variety of different diets for different species of *Spodoptera*, particularly *S. exigua* (Shorey & Hale, 1965; Singh & Moore, 1985; Elvira *et al.*, 2010) (Table 1). To evaluate the effects of different dietary protein sources on the insect biology, different dry seed powders were used in each diet, including; soybean chunks, chickpea, kidney bean, mung bean, pinto bean, white bean, lentil and corn. Similar to the protocol of Shorey & Hale (1965), a combination of methyl 4-hydroxybenzoate, sorbic acid, and formaldehyde was used for mold inhibition. The diets consisted of three fractions (A-C; Table 1). The ingredients of fraction A were mixed completely. Then, the agar was boiled in distilled water with intermittent shaking until dissolved thoroughly and after reaching to 90 °C, methyl 4-hydroxybenzoate was added. Consequently, this mixture (fraction B) was poured into prepared fraction A. Finally, when the mixture of fractions A and B was cooled to 60 °C, the ingredients of fraction C (consisting of the antimicrobial agents and vitamins) were mixed properly with them. After blending all ingredients together, each diet was poured into sterile containers of 400 ml (18 cm × 12 cm × 3 cm), allowed to solidify at room temperature and kept at 4 °C until used.

### Bioassays procedure

To study the beet armyworm biology, 45 freshly emerged neonates from a laboratory colony were transferred to 50 ml airtight plastic cups. Blocks of different artificial diets (1 cm × 1 cm × 1 cm) were placed in each cup and replaced at 2-d intervals.

**Table 1.** Composition of the artificial diet used for rearing *Spodoptera exigua*

Fractions	Ingredients	Quantity
Fraction A	Dry seed powder	96 g
	Wheat germ	20 g
	Brewer's yeast	22 g
	Distilled water	350 ml
	Sunflower oil	3 ml
Fraction B	Agar	7.4 g
	Distilled water	250 ml
	Methyl 4-hydroxybenzoate	2.2 g
Fraction C	Ascorbic acid	2.7 g
	Sorbic acid	1 g
	Formaldehyde (37%)	1.4 ml
	Vitamin mixture consisting of vitamin A (5000 I.U.), D <sub>3</sub> (400 I.U.), C (60 mg), B <sub>1</sub> (1.5 mg), B <sub>2</sub> (1.7 mg), B <sub>6</sub> (2 mg), B <sub>12</sub> (6 mcg), E (30 I.U.), nicotinamide (20 mg), folic acid (0.4 mg), iron (18 mg), calcium (125 mg), iodine (0.15 mg) and magnesium (100 mg)	1 g

To prevent cannibalism, after reaching the larvae to 3<sup>rd</sup>. instar (seven days later), each survived larva was kept individually in a separate cup until pupation. The cups related to each treatment were placed on separate trays. The number of surviving larvae on 7th and 14th days, larval developmental time and pupation per-centage (% pupa/neonate larvae) were recorded. The resulting pupae were taken out of the diet and collected in Petri dishes. Pupae were sexed using stereomicroscopy and their weight (0-24 h post-emergence), duration of pupal stage and adult emergence per-centage (% adults/pupa) were calculated accordingly. Five pairs of newly emerged adults from the same diet treatment were selected and transferred to transparent plastic containers (8.5 cm diameter, 6.5 cm height) with air-permeable caps. These oviposition boxes were wrapped inside with a white polypropylene non-woven filter cloth to facilitate egg laying and contained 10% honey-soaked cotton balls in the lid of a bottle for the adult feeding. Three oviposition boxes were prepared for each treatment. Cloths containing eggs were collected daily and transferred to the rearing cups. The adult's longevity, total number of eggs laid per female (fecundity) and percentage of hatched eggs (fertility) were also counted at 24-h intervals. Insects were maintained in the before mentioned conditions. Each treatment had five replications.

After recording data and analyzing the results, chickpea and soybean chunks flour (which showed best results) were tested again in another experiment. Three treatments: 1) soybean chunks, 2) chickpea, and 3) mix of soybean chunks and chickpea (50:50) used in separate diets. All cited parameters and indices were recorded. Each treatment had five replications.

### Growth and Fitness indices

Larval, pupal and overall immature growth indices, standardized growth indices, and fitness indices of *S. exigua* on various artificial diets were calculated using the following formulas (Amer & El-Sayed, 2014);

$$\begin{aligned} \text{Larval growth index} &= \frac{\text{Pupation (\%)}}{\text{Larval period (days)}} \\ \text{Pupal growth index} &= \frac{\text{Emergence (\%)}}{\text{Pupal period (days)}} \\ \text{Immature growth index} &= \frac{\text{Emergence (\%)}}{\text{Larval period} + \text{Pupal period (days)}} \\ \text{Standardized growth index} &= \frac{\text{Pupal weight (g)}}{\text{Larval period (days)}} \\ \text{Fitness index} &= \frac{\text{Pupation (\%)} \times \text{Pupal weight (g)}}{\text{Larval period (days)} + \text{Pupal period (days)}} \end{aligned}$$

### Statistical analysis

To evaluate the effects of different artificial diets on the insect biological parameters, growth and fitness indices, data were subjected to statistical analysis using one-way ANOVA. Comparisons of mean  $\pm$  SE were carried out using Duncan's multiple range test and data analysis was done using SPSS version 22.0. (IBM ©). Excel was used for creating graphs.

### Results

Biological parameters of *S. exigua* on eight different artificial diets were shown in Table 2. Chickpea, soybean and white bean diets had the highest larval survival at the 7th day post-hatch ( $F_{7,39}=88.407$ ,  $P<0.01$ ). On the 14th day, highest larval survival was recorded on the chickpea and white bean, followed by soybean chunks, mung bean and kidney bean ( $F_{7,39}=83.735$ ,  $P<0.01$ ). The lowest larval survival was observed on lentil, pinto bean and corn and no larvae pupated on these diets. The shortest larval period was recorded on soybean chunks, chickpea and white bean ( $F_{4,24}=12.671$ ,  $P<0.01$ ). However, percentage of pupation was highest only in soybean chunks and chickpeas, and their values were significantly different from white bean ( $F_{4,24}=14.705$ ,  $P<0.01$ ). The highest female pupa weight was on chickpea ( $F_{4,24}=5.662$ ,  $P<0.01$ ) and the highest male pupa weight was on chickpea and soybean chunks ( $F_{4,24}=5.417$ ,  $P<0.01$ ). Female pupae tended to be heavier than males on all diets. The duration of the pupa was shortest on chickpea, soybean and white bean for both male ( $F_{4,24}=8.334$ ,  $P<0.01$ ) and female ( $F_{4,24}=5.417$ ,  $P<0.01$ ). Pupal period was longer in males than in females on all diets. The percentage of the pupa which transferred to the adult stage was highest on soybean chunks and chickpea diets ( $F_{4,24}=7.007$ ,  $P<0.01$ ). Adult longevity was longer for insects reared on soybean, kidney bean and chickpea diets than insects fed on mung bean and white bean diets ( $F_{4,24}=6.794$ ,  $P<0.01$ ). The average number of eggs laid by females on soybean and chickpea was greater than others ( $F_{4,14}=9.105$ ,  $P<0.01$ ).

**Table 2.** Biological parameters (Mean  $\pm$  SE) of *Spodoptera exigua* on eight different artificial diets under laboratory conditions

Biological parameters	Different artificial diets							
	Chickpea	Corn	Kidney bean	Lentil	Mung bean	Pinto bean	Soybean chunks	White bean
Larval survival (%)								
7th day	100 $\pm$ 00a	45.78 $\pm$ 1.5 e	91.11 $\pm$ 0.99b	81.78 $\pm$ 2.37c	95.11 $\pm$ 1.08ab	74.67 $\pm$ 3.18d	97.78 $\pm$ 0.99a	94.67 $\pm$ 2.86ab
14th day	88.89 $\pm$ 4.33a	32.00 $\pm$ 3.69e	65.33 $\pm$ 3.95d	16.00 $\pm$ 2.47f	69.78 $\pm$ 3.48cd	23.56 $\pm$ 1.50ef	78.22 $\pm$ 2.66bc	81.78 $\pm$ 1.63ab
Larval period (days)	14.15 $\pm$ 0.28a	-	16.29 $\pm$ 0.37b	-	15.84 $\pm$ 0.43b	-	13.52 $\pm$ 0.09a	14.4 $\pm$ 0.37a
Pupation (%)	68.44 $\pm$ 5.31a	-	45.78 $\pm$ 4.25b	-	36.89 $\pm$ 0.89b	-	69.78 $\pm$ 3.48a	41.33 $\pm$ 5.56b
Pupal weight (mg)	93.84 $\pm$ 2.93a	-	71.42 $\pm$ 5.36b	-	77.42 $\pm$ 3.87b	-	82.62 $\pm$ 1.71b	75.56 $\pm$ 3.23b
♀ ♂	87.51 $\pm$ 1.65a	-	67.86 $\pm$ 3.08c	-	72.62 $\pm$ 5.84bc	-	82.45 $\pm$ 0.49ab	72.74 $\pm$ 3.68bc
Pupal period (days)	7.67 $\pm$ 0.14a	-	9.25 $\pm$ 0.22b	-	9.03 $\pm$ 0.4b	-	8.12 $\pm$ 0.11a	8 $\pm$ 0.32a
♀ ♂	8.52 $\pm$ 0.13a	-	9.58 $\pm$ 0.33b	-	9.6 $\pm$ 0.20b	-	8.56 $\pm$ 0.08a	8.45 $\pm$ 0.20a
Adult emergence (%)	82.87 $\pm$ 5.07ab	-	69.54 $\pm$ 3.62 bc	-	67.44 $\pm$ 4.84 c	-	90.47 $\pm$ 5.22a	58.64 $\pm$ 4.99c
Adults longevity (days)	7.73 $\pm$ 0.20ab	-	8.1 $\pm$ 0.66ab	-	7.25 $\pm$ 0.49b	-	9.37 $\pm$ 0.13a	5.24 $\pm$ 0.96c
Fecundity (eggs/♀)	440 $\pm$ 99ab	-	215 $\pm$ 31cd	-	326 $\pm$ 42bc	-	518 $\pm$ 58a	84 $\pm$ 20d
Fertility (%)	80.96 $\pm$ 4.26ab	-	69.95 $\pm$ 8.51 bc	-	88.02 $\pm$ 0.82 a	-	75.61 $\pm$ 4.96 ab	56.34 $\pm$ 2.24c
Incubation period (days)	3.00 $\pm$ 00a	-	3.00 $\pm$ 00a	-	3.00 $\pm$ 00a	-	3.00 $\pm$ 00a	30.0 $\pm$ 00a
Life span (days)	27.04 $\pm$ 0.33a	-	30.71 $\pm$ 0.56b	-	30.16 $\pm$ 0.50b	-	26.86 $\pm$ 0.11a	27.62 $\pm$ 0.49 a

The means followed by different letters in the same rows are significantly different ( $P < 0.01$ ) based on Duncan's test.

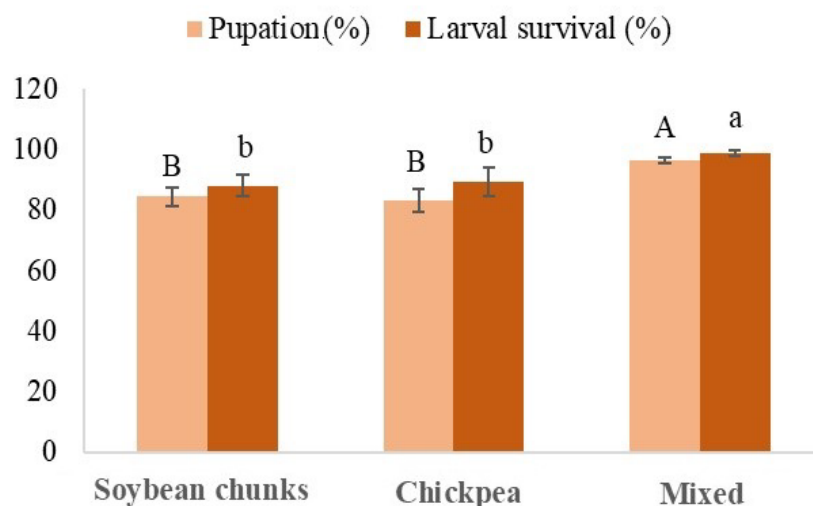
Similarly, the proportion of eggs that hatched was highest on these two diets and mung bean ( $F_{4,14}=5.956$ ,  $P<0.01$ ). The incubation period was constant three days in all five treatments. Finally, insects from larvae reared on soybean, chickpea and white bean diets completed their life cycle in a shorter time than insects reared on mung bean and kidney bean diets ( $F_{4,24}=17.15$ ,  $P<0.01$ ).The suitability of various diets was evaluated using growth and fitness indices at different stages. Different diets significantly ( $P<0.01$ ) affected larval growth, pupal growth, immature growth, standardized growth and fitness indices of *S. exigua* (Table 3). Among the five different diets that allowed larvae to complete their life cycle, the larval growth index was greatest for soybean showing no significant difference from chickpeas, while they were significantly greater than white bean, kidney bean and mung bean values ( $F_{4,24}=15.558$ ,  $P<0.01$ ). Also, the pupal growth index of *S. exigua* in soybean chunks and chickpea diets was higher than the other three diets ( $F_{4,24}=9.962$ ,  $P<0.01$ ). Similar results were also found in immature growth index ( $F_{4,24}=11.347$ ,  $P<0.01$ ), standardized growth index ( $F_{4,24}=10.887$ ,  $P<0.01$ ) and fitness index ( $F_{4,24}=18.827$ ,  $P<0.01$ ).

According to the results, soybean chunks and chickpea diets were the best ingredients to make an artificial diet for *S. exigua*. Therefore, these two flours were mixed as fifty in separate diet and all biological parameters and indices were compared to larvae fed on soybean chunks and chickpea diets separately. No significant differences were observed among the biological parameters of the three different treatments, except for larval survival and pupation rates. As shown in Fig. 1, the mixed diet (soybean chunks - chickpea diet) increased larval survival ( $F_{2,14}=3.977$ ,  $P<0.05$ ) and pupation rate ( $F_{2,14}=4.667$ ,  $P<0.05$ ). Data on other biological parameters did not given. Furthermore, no significant differences were observed between the indices of the three different feeding insects except the larval growth index and the fitness index. As shown in Fig. 2a, the larval growth index ( $F_{2,14}=5.953$ ,  $P<0.05$ ) of larvae fed on the mixed diet was significantly higher than the soybean chunks and/or chickpea diets. Also, the fitness index ( $F_{2,14}=4.105$ ,  $P<0.05$ ) of larvae fed on mixed diet is significantly different from larvae fed on soybean chunks diet (Fig. 2b).

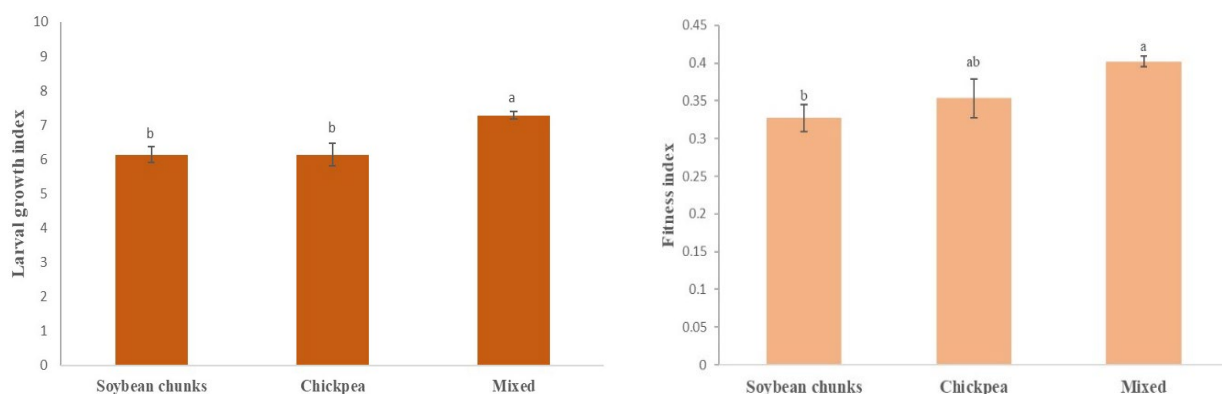
**Table 3.** Growth and fitness indices (Mean  $\pm$  SE) of *Spodoptera exigua* on five different artificial diets under laboratory conditions

Indices	Different artificial diets				
	Chickpea	Kidney bean	Mung bean	Soybean chunks	White bean
Larval growth index	4.86 $\pm$ 0.40a	2.83 $\pm$ 0.29b	2.34 $\pm$ 0.11b	5.16 $\pm$ 0.27a	2.91 $\pm$ 0.45b
Pupal growth index	10.21 $\pm$ 0.51a	7.40 $\pm$ 0.41b	7.29 $\pm$ 0.68b	10.87 $\pm$ 0.68a	7.10 $\pm$ 0.53b
Immature growth index	3.72 $\pm$ 0.21a	2.71 $\pm$ 0.17b	2.69 $\pm$ 0.23b	4.14 $\pm$ 0.25a	2.58 $\pm$ 0.19b
Standardized growth index	0.0064 $\pm$ 0.00014a	0.0043 $\pm$ 0.0003c	0.0048 $\pm$ 0.0004bc	0.0061 $\pm$ 0.00011a	0.0052 $\pm$ 0.0003b
Fitness index	0.28 $\pm$ 0.022a	0.13 $\pm$ 0.017b	0.11 $\pm$ 0.008b	0.26 $\pm$ 0.016a	0.14 $\pm$ 0.024b

The means followed by different letters in the same rows are significantly different ( $P < 0.01$ ) based on Duncan's test.



**Fig. 1.** Larval survival and pupation percentage (Mean  $\pm$  SE) of *Spodoptera exigua* on three different artificial diets under laboratory conditions. The means followed by different letters on the columns are significantly different ( $P < 0.05$ ) based on Duncan's test.



**Fig. 2.** Larval growth index (right) and fitness index (left) (Mean  $\pm$  SE) of *Spodoptera exigua* on three different artificial diets under laboratory conditions. The means followed by different letters on the columns are significantly different ( $P < 0.05$ ) based on Duncan's test.

As it is obvious, larval survival rate for soybean chunks diet (88%) and the pupation percentage for soybean chunks diet (84.44%) and chickpea diet (83.11%) in these second bioassays were greater than in the first bioassay. The reason for the reduced larval mortality and percentage of pupation is that the larvae lose a lot of body weight during the pre-pupa stage, and the presence of fresh food in the rearing container at this stage, increases the relative humidity inside the rearing containers and causes the larvae to suffocate. Soybean chunks, in particular, can hold large amounts of dietary water, increasing larval mortality due to suffocation at the final instar. Therefore, in the second bioassay, fresh food was avoided on the last day of the larval stage, which increased the larval survival and the percentage of pupation.

## Discussion

Mass rearing of insects is a basic pre-requisite for conducting sterile insect techniques (SIT) assessments and  $F_1$  sterility principle (Seth & Sharma, 2002). The current investigation was aimed to compare the biology of *S. exigua* in artificial diets with different protein-based ingredients previously reported in the literature.

Numerous techniques have already been developed using soy protein in artificial diets for rearing the larvae of noctuid species, including *S. exigua*. Actually, using soy components in insect diets was mentioned in Western literature since the 1960s. The use of soybeans in the diet of the Asian mulberry *Bombyx mori* (L.) (Lepidoptera: Bombycidae) was used for at least several decades before being adopted by Western researchers. The adoption of soy flour was the most important improvement in mass-rearing systems (Cohen, 2018). Srinivasa Murthy et al. (2006) used soybean flour to prepare diets for *S. exigua*. They found a larval period of 16.3 days, a pupal period of 9.9 days, a pupation of 70.9%, an adult emergence rate of 68.8%, an adult longevity of 9.9 days and a fecundity of 197 eggs per female on a soya flour diet. All these data are in accordance with our data, and the current study also



yields a more reasonable values for *S. exigua* biological and reproductive parameters using soyabean chunks in the larval diet. Elvira *et al.* (2010) claimed that soy protein is an inexpensive protein source that provides all the essential amino acids without affecting the physiological functions of *S. exigua*. They used soy protein and wheat germ in their diet, to rear *S. exigua*. However, in contrast with this study, their diet contained higher levels of wheat germ and less soy protein. Zhang *et al.* (2011) used corn, soybean and wheat powders in a diet for rearing *S. exigua*. They compared different levels of protein and carbohydrate contents (yeast or glucose: cellulose) in the diet on insect performance. They claimed that with increased protein and soluble carbohydrate content, development, fecundity and population growth index increased. Also, larval development period, pupal period and sum of larval, pupal and adult periods all decreased. Di *et al.* (2021) assessed the effects of a natural diet (tobacco and Chinese cabbage) and an artificial diet based on the soybean meal on the population parameters of the tobacco caterpillar, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). They observed the shortest developmental time and highest fecundity in individuals fed the artificial diet. Similarly, in current research, soy protein is one of the best ingredients to make an optimized diet for rearing *S. exigua*. Naghdi & Bandani (2013) compared the growth and development of sugar beet armyworm on an artificial diet contained wheat germ and casein protein. They reported pupal period, adult longevity and pupal weight of 8.56 and 10.33 days and 57 mg, respectively. All data recorded in the current study were better on soybean chunks and chick pea diets. Also, Elvira *et al.* (2010) claimed that soya protein is cheaper and more efficient than a casein-based diet in rearing of *S. exigua*. When soybean flour was substituted for casein in the wheat germ diet of the tobacco bud worm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), larval growth was equal to that of the casein-containing diet (Shaver & Raulston, 1971).

Besides, soy protein, other beans and grains are used in *Spodoptera* spp. artificial diets. Shorey & Hale (1965) found no significant differences in survival of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) when was reared on the diets contained several varieties of *Phaseolus* spp.; including kidney beans, navy beans, lima beans, and pinto beans. So, they selected the least expensive variety available in southern California (pinto beans) for rearing of nine important lepidopteran species, including *S. exigua*. However, the current study examining the effects of different beans such as kidney beans, white beans, and pinto beans on the beet armyworm survival, found significant differences between varieties. Abdullah *et al.* (2000) used mung bean seeds to provide protein to an artificial diet of *S. exigua* and stated that the biological composition of insects fed this diet was better than those fed soybean leaves. They recorded 578 eggs per female and 53.18% hatching for *S. exigua* on a diet containing mung bean. Also, we found 326 eggs per female with 88% fertility on a similar diet. They also found 15.7, 6.73, 6.29 and 25.43 days for larval, pupal, adult and egg to adult emergence periods, respectively, and a pupae weight of 78.70 mg. All these data are in accordance with data of the current study resulted in mung bean diet (except the pupal period). Alfazairy *et al.* (2012) used lentil and rice flour to prepare an artificial diet for the Egyptian cotton leafworm, *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae), but in current study, larvae of *S. exigua*, which feed on an artificial diet containing dry lentil flour, could not complete their life cycle. Shorey & Hale (1965) recorded 89% larval survival, 94% pupal survival, and 44% survival of egg to adult in *S. exigua* when fed pinto bean contained artificial diet, however in the current study larva fed on similar diet failed to complete the larval period and progress to the pupal stage.

Nair *et al.* (2019) used chickpea powder to rear six species of lepidoptera, including beet armyworm. In the current study, chickpea is also reported as the best legume flour for preparing an artificial diet for *S. exigua*. They reported 13.2 days larval period, 87.2% larval survival, 87.2% pupation, 7.5 days pupal period, 80 mg pupal weight, 92.3% adult emergence, 7.3 days adult longevity, 1030 eggs per female and 93.5% egg hatching. When compared with the data obtained in the current study from a diet containing chickpeas, these values are all consistent with our data, except for higher adult emergence and fecundity.

Similar to this research, Hemati *et al.* (2013) and Amer & El-Sayed (2014) calculated the growth and fitness index of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on different host plants and stated that these indices differed significantly, and claimed that lepidoptera larvae feed on high nutrient food increase growth rates and complete developmental time faster than those fed on low quality food. Gupta *et al.* (2005) using an artificial diet containing wheatgerm, kidney bean and chick pea flour, found larval, pupal and total immature growth indices of *S. litura* 4.31, 9.92 and 2.84, respectively. Also, Mardani-Talaei *et al.* (2014) reported the highest growth indices of larva and pupa of *S. exigua* on different corn hybrids, 6.07 and 9.66, respectively. These indices are in accordance with our data obtained from soybean chunk and chickpea diets. In addition, Di Bello *et al.* (2017) said that the fitness index of the unbarred *Spodoptera* moth *S. albula* (Walker) (Lepidoptera: Noctuidae) was significantly different when fed on three artificial diets. These three artificial diets contained different amounts of saccharose, soy protein, wheat germ, brewer yeast, casein and beans as proteins or carbohydrates. The diet containing all these ingredients except saccharose was the most suitable for *S. albula*.

Lee *et al.* (2008) claimed that dietary protein quality is an important determinant for insect rearing and *S. littoralis* larvae fed a high-quality protein diet have higher survival and faster growth rates than larvae on a low-quality protein diet; they also have more heavily melanized cuticles and higher antibacterial activity. Merckx-Jacques

et al. (2008) claimed that high mortality and developmental delay were observed on the high-carbohydrate and low-protein diets, highlighting the potential detrimental effects of excess carbohydrates and the need for protein for growth and development in *S. exigua*. The current study also showed that larvae were unable to transition to pupa on a diet containing corn flour instead of beans, demonstrating the importance of bean flour as protein-rich sources.

Truzzi et al. (2021) used three artificial diets to rear the fall armyworm *S. frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae); 1) based on bean, wheat germ and brewer's yeast; 2) containing half the original amount of protein, and 3) with twice the original amount of protein. They claimed that varying levels of protein in the diet did not negatively influence population growth. Pinto et al. (2019) stated that between three artificial diets; a standard diet based on beans and wheat germ, a diet with beans and corn flour, and a diet with green corn and wheat germ, the most suitable diets for rearing *S. frugiperda* in the laboratory are first and last ones. This demonstrates the importance of wheat germ in the diet which we used in all studied diets for rearing *S. exigua*. Wheatgerm is a major component of diets containing substances that stimulate the feeding response of insects and is thought to be a source of energy, minerals and lipids (Vanderzant et al., 1962). However, Jin et al. (2020) compared life performance of *S. frugiperda* on soybean and sucrose, soybean and wheat germ, chickpea and wheat germ, and corn and soybean based four artificial diets and claimed the last diet was the most suitable diet for this insect mass rearing.

Karimi-Malati et al. (2012) recorded the shortest duration of larval and pupal stages of *S. exigua* on different sugar beet cultivars, 14.55 and 8.81 days, respectively. In another study, Karimi-Malati et al. (2014) found these values 14.42 and 9.78 days at 25 °C in sugar beet leaves. These are consistent with our data on artificial feeding of soybean chunks, chickpea and white bean. Therefore, the use of these artificial diets does not prolong the duration of the immature stages.

Seth & Sharma (2002) compared the growth, development, and reproductive performance of *S. litura* grown on various diets (castor leaves, and semi-synthetic diets; chickpea, wheat, wheatgerm and soybean). Like our results, they found chickpea and soybean diets as the optimal diets, along with castor leaves. Pupation, larval period, life span, adult emergence, growth index, fertility and adult longevity were greatest on both chickpea, and soybean diets, with no significant difference between them. Also, on par with our results, the female pupae on the chickpea diet gained the highest body weight. In contrast to current results, mean oviposition was highest for females that developed on the chickpea diet. As a whole, they claimed the chickpea diet containing sinigrin was the most suitable as a phagostimulant for *S. litura* rearing.

## Conclusion

Through the current study, the survival and developmental parameters of *S. exigua* were investigated using eight artificial diets. The lowest larval survival rates were observed on lentil, pinto bean and corn, and pupation did not occur on these diets. Among the data resulting from the other five artificial diets, the best developmental and reproduction values and growth and fitness indices were recorded on soybean chunks and chickpea diets. Generally, results showed that except for female pupa weight, no significant differences were seen between soybean chunks and chickpea diets. Both ingredients are the cheapest beans on the market of Iran. These findings therefore indicate that a diet containing soybean chunks and chickpea flour is the optimal medium for *S. exigua* rearing. These results may offer a theoretical basis for mass rearing of this insect pest.

## Statements and Declaration

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# بررسی مقایسه‌ای اثر جیره‌های مصنوعی مختلف بر روی مولفه‌های زیستی کرم برگ‌خوار چغندرقد *Spodoptera exigua* (Lepidoptera: Noctuidae)

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## چکیده

کرم برگ‌خوار چغندرقد (*Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)، حشره‌ای چندخوار و آفت بسیاری از گیاهان زراعی و سبزیجات در بیش‌تر نقاط جهان است. جهت بررسی روش‌های مختلف کنترل آفات، مانند روش نرغیمی (SIT)، پرورش مستمر جمعیت گونه‌های مختلف حشرات در شرایط آزمایشگاهی بر روی رژیم‌های غذایی مصنوعی، حائز اهمیت است. هدف از تحقیق حاضر، بررسی اثرات رژیم‌های غذایی مبتنی بر پودر مواد پروتئینی مختلف (پروتئین سویا، نخود، لوبیا، ماش، لوبیا چیتی، لوبیا سفید، عدس و ذرت) بر شاخص‌های زیستی و رشدی این حشره بود. بدین منظور، جیره‌های غذایی مصنوعی مختلف تهیه گردیدند و لاروها بر روی این جیره‌ها پرورش داده شدند. میزان بقای لاروی، طول دوره لاروی، درصد شفیره شدن، وزن شفیره‌ها، طول دوره شفیرگی، درصد ظهور حشرات کامل، طول عمر حشرات کامل، زادآوری، باروری و طول کل دوره رشدی، مورد محاسبه قرار گرفت. نرخ بقای لاروی در عدس، لوبیا چیتی و ذرت کم‌ترین بود ( $P < 0.01$ ) و شفیره در این جیره‌ها تشکیل نشد. در میان داده‌های حاصل از پنج رژیم غذایی مصنوعی دیگر، بیش‌ترین و بهترین مولفه‌های زیستی مورد مطالعه، میزان نشو و نما، تولید مثل و شاخص‌های رشدی، روی رژیم‌های حاوی پروتئین سویا و نخود ثبت شد ( $P < 0.01$ ). هنگامی که پودر پروتئین سویا و نخود به نسبت ۵۰ به ۵۰٪ با هم مخلوط شدند، میزان بقای لاروی، درصد شفیره شدن، شاخص رشدی لارو و شاخص تناسب رشدی، حتی بهتر از آزمایش قبل ثبت شدند. در نتیجه، پروتئین سویا و نخود به عنوان بهترین ترکیبات پروتئینی آسیاب شده خشک در جیره مصنوعی کرم برگ‌خوار چغندرقد معرفی می‌گردند.

**کلمات کلیدی:** جیره مصنوعی، کرم برگ‌خوار چغندرقد، مولفه‌های زیستی، شاخص‌های رشدی، کنترل آفات

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