An Economically Modified Semi-Synthetic Diet for Mass Rearing the Egyptian Cotton Leaf Worm Spodoptera littoralis


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Abstract: This work was aimed to evaluate a simplified semi-synthetic diet based on combination of starch/agar for mass rearing of Spodoptera littoralis. Two modified semi-synthetic diets (A & B) proved successful in maintaining the culture of S. littoralis for five successive generations without apparent adverse effects throughout the insect stages. The mean values of tested biological parameters were determined on the reared insects fed on either of the modified diets. The results showed a higher larval weight, pupal weight, adult emergence, fertility and development index as compared to the agar-based standard diet of Shorey and Hale (1965). Whereas, percentage pupation, survival, fecundity and sex ratio were non-significantly different in the tested treatments. Moreover, the cost of ingredient reduced by 45.6% and 33.3% per one liter of diets A and B, respectively as compared to the agar-based standard diet. The obtained results provide an economically viable diet for mass rearing, thus promising for large-scale production of S. littoralis laboratory culture.

Key words: Economical semi-synthetic diet • Biological parameters • Spodoptera littoralis

INTRODUCTION

The Egyptian cotton leaf worm, Spodoptera littoralis (Boisd.) is a polyphagous and widely distributed pest on many vegetable, field, ornamental crops and several other economic host plants. It is important to be able to economically rear important insects to study their life history, behavior, feeding habits and their susceptibility and resistance to chemical and biological pesticides. The development of artificial diets, pioneered by Vanderzant et al. [1], facilitated the continuous production of insects. So far various artificial diets have been developed and proposed for the maintenance and continuous rearing of economically important insects [2-7]. Since then, numerous species of dipterans, lepidopterous and coleopterans have been successfully reared under controlled laboratory conditions [8-14]. Developments of rearing larvae of S. littoralis on artificial diets have been investigated by many authors [15-20]. Rearing insects on artificial diets is an expensive process; so considerable effort has been invested in the development of low cost-effective artificial diets based on the use of cheaper ingredients and testing potential agar substitutes [10, 21-26]. The present study is aimed to develop a low-cost simplified diet based on combination of starch/agar instead of agar for mass rearing of the cotton leaf worm Spodoptera littoralis larvae under laboratory conditions.

MATERIALS AND METHODS

Test Insect: A disease-free culture of S. littoralis was successfully maintained for several generations in the insectary under controlled conditions of 26±2°C, 65-70 % RH and 16L: 8D photoperiod throughout the duration of study. The larval stages were fed on a semi-synthetic diet described by Shorey and Hale [15] which is used as a standard control diet.

Modification of Diets for Rearing S. littoralis: Two modifications on the standard semi-artificial diet developed by Shorey and Hale [15] mainly constituted by substitution of agar with starch. The ingredients of the standard diet [15] and both the two modified diets per one kilogram haricot beans (Phaseolus spp.) are listed in Table 1.
Preparation of Modified Diet: The dry beans soaked in tap water overnight before use and autoclaved at 120°C for 20 min. After cooking, all the ingredients except the ascorbic acid, formaldehyde and agar were placed, in an electric blender with 50% of total volume of water. At the same time the agar (or starch) was separately dissolved in the remaining amount of water, autoclaved then mixed with the other blending ingredients. When the diet temperature cooled to less than 60°C, ascorbic acid and formaldehyde were added and well mixed. Immediately, the diet was poured in sterilized boxes, before it turned into semi-solid and left to cool. The diet could be stored in a refrigerator until required.

Rearing Procedure

Adult Stage: Twenty couples of *S. littoralis* newly emerged adult moths (*♂ + ♀*) were kept in a cylindrical mating cage (11 cm in diameter and 22.5 cm in length). Mating and egg-lying took place in the same cages which were lined with a white tissue paper as an oviposition site. A piece of cotton filter soaked in 10% solution of honey was provided for feeding the moths. The tissue papers containing egg-masses were daily removed, labeled and replaced with new ones.

Egg Stage: Newly deposited egg-masses were sterilized by formalin vapor 5% for 20-30 minutes [27]. The eggs were placed in a plastic rectangular container containing a square of diet until hatching. A small piece of wetted cotton wool was placed on the container as a humidity source to facilitate egg hatch.

Larval Stage: Hatching usually occurs in three days and the neonates larvae were placed in a clean sterilized container (17 x 17 x 8 cm) using a small Camel’s hair brush and left until reached 4th instars. The box’s lid was perforated and sub-covered with a fine cheese cloth mesh to permit adequate gaseous exchange to occur and provide a suitable surface for attachment of larvae during molting [28]. Larvae were then transferred and divided into new containers with fresh diet as necessary. When they reached 6th instars, the containers were lined with a 0.5 cm layer of soft sawdust for pupation.

Pupal Stage: At the end of larval stage, the pupae were grouped according to their formation dates, then sterilized and transferred into clean sterilized boxes (16 x 16 x 8.5 cm) containing a 2 cm deep layer of soft sawdust moistened with distilled water until adult emergence.

Evaluation of Tested Diets: The different biological parameters of *S. littoralis* reared on tested media were studied through five successive generations under the same conditions. Newly-hatched larvae from a single egg mass were gently transferred (using a small Camel’s hair brush) into plastic pots containing the tested diet, one hundred neonates were divided into four groups of 25 neonates each and used for diet evaluation test. The artificial diet of Shorey and Hale [15] was served as standard control diet. Three-day old larvae were weighed until they reached the 7th day; then transferred to small plastic polypots containing a piece of the same diet. A small piece of tissue paper was sub-covered the lid of the polypot and renewed when necessary to absorb excess moisture from the food and feces. The weights of individual larvae were recorded until reaching prepupae. Pupae were sexed, weighed, sterilized and transferred to sterilized polypots provided with tissue paper until moth emergence. Fecundity, fertility and longevity evaluations were carried out on freshly emerged couples placed in a small cylindrical cage (8 cm in diameter and 13 cm in length) and held at the same previously described conditions. The tissue papers containing egg-masses were daily removed, labeled, calculated and replaced with new ones. From these observations, the growth and developmental indices were calculated as follows [14]:

\[
\text{Larval growth index} = \frac{\% \text{ Pupation}}{\text{Larval Period (days)}}
\]

\[
\text{Pupal growth index} = \frac{\% \text{ Adult emergence}}{\text{Pupal Period (days)}}
\]

\[
\text{Total developmental index} = \frac{\% \text{ Survival}}{\text{Total Developmental Period (days)}}
\]

Statistical Analysis: Statistical analysis of obtained data was conducted using Pro. GLM in SAS [29]. Means separation was conducted using Duncan multiple range test in the same program.
RESULTS AND DISCUSSION

The obtained results shown in Table 2 and Fig. 1 represent the mean larval weight of 10- and 12-day-old larvae reared after hatching on the standard and the two modified diets. The recorded data were (191.94±104.61 and 739.05±724.26 mg) for the standard diet and (249.16±99.35 and 935.67±283.51 mg) for the diet (A), whereas (208.93±101.51 and 892.97±209.36 mg) for the diet (B), respectively. An opposite trend was observed, where the mean pupal weight was higher on diet (B) (386.47±69.48 mg) than that reared on the standard diet (323.47±72.14 mg) and diet (A) (366.40±95.84 mg) (Table 2).

The survival of larvae fed on both two modified diets A and B was estimated by (97.03±2.53% and 98.23±2.73%, respectively), also similar to the larvae reared on the standard diet (97.22±3.02%). The result also showed that no significant differences were observed in the percentage of pupation on all tested diets (Table 3). About 95% neonates completed their developmental cycle and emerged as normal adults on either of the two modified diets compared to 88.85% recorded for the standard control diet, with no significant differences between diets in the percentage of malformed adults (Table 3). The highest value of 4.33% was recorded for standard diet.

The results shown in Table 4 demonstrated that the diet A provided a faster development of the insect, with a mean larval period of (13.78 days) and a mean total developmental period of (22.2 days) significantly lower than those observed in the standard diet (14.15 and 22.6 days, respectively) and diet B (14.19 and 22.4 days, respectively) (Table 4). The mean pupal periods on modified diets (8.4% for Diet A and 8.2% for Diet B) were also similar to that of insects reared on standard diet (8.4%) with no significant differences (Table 4).

The obtained results showed that, adult longevity of males and females was longer for insects fed and reared on either of the two modified diets compared with those reared on the standard diet (Fig. 2).

Table 2: Mean larval weight in mg of 10- and 12-day-old larvae after hatching (means ± STD) and mean pupal weight in mg (means ± STD) of S. littoralis reared on the standard and two modified diets

<table>
<thead>
<tr>
<th>Tested diet</th>
<th>Larval weight (10-day-old) (mg)</th>
<th>Larval weight (12-day-old) (mg)</th>
<th>Pupal weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>191.94±104.61 b</td>
<td>739.05±274.26 b</td>
<td>323.47±72.14 c</td>
</tr>
<tr>
<td>Diet A</td>
<td>249.16±99.35 a</td>
<td>935.67±283.51 a</td>
<td>366.40±95.84 b</td>
</tr>
<tr>
<td>Diet B</td>
<td>208.93±101.51 b</td>
<td>892.97±209.36 a</td>
<td>386.47±69.48 a</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (P <0.05)

Table 3: Percentage of survival, pupation, adult emergence and malformed adults of S. littoralis reared on the standard and both the two modified diets

<table>
<thead>
<tr>
<th>Tested diet</th>
<th>Survival (%)</th>
<th>Pupation (%)</th>
<th>Adult emergence (%)</th>
<th>Malformed adults (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>97.22±3.03 a</td>
<td>96.14±2.47 a</td>
<td>88.85±7.36 b</td>
<td>4.33±3.91 a</td>
</tr>
<tr>
<td>Diet A</td>
<td>97.03±2.53 a</td>
<td>97.50±2.61 a</td>
<td>95.09±5.56 a</td>
<td>2.89±2.71 a</td>
</tr>
<tr>
<td>Diet B</td>
<td>98.23±2.73 a</td>
<td>98.04±1.96 a</td>
<td>95.70±3.65 a</td>
<td>1.78±1.79 a</td>
</tr>
</tbody>
</table>

Means followed by identical letters are not significantly different for comparisons between treatments within each column (P <0.05)

Table 4: Mean larval, pupal and total developmental period in days of S. littoralis reared on the standard and both the two modified diets

<table>
<thead>
<tr>
<th>Tested diet</th>
<th>Larval period (days ± STD)</th>
<th>Pupal period (days ± STD)</th>
<th>Total developmental period (days ± STD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>14.149±1.18 a</td>
<td>8.449±1.06 a</td>
<td>22.598±1.06 a</td>
</tr>
<tr>
<td>Diet A</td>
<td>13.778±1.05 b</td>
<td>8.437±1.18 a</td>
<td>22.215±1.03 b</td>
</tr>
<tr>
<td>Diet B</td>
<td>14.191±0.81 a</td>
<td>8.225±1.08 a</td>
<td>22.416±0.87 a</td>
</tr>
</tbody>
</table>

Means followed by identical letters are not significantly different for comparisons between treatments within each column (P <0.05).
Fig. 2: Adult longevity in days of S. Littoralis moths obtained from larvae reared on standard and modified diets. Columns headed by identical letters are not significantly different for comparisons of diet treatment within each sex (P=0.05)

Thus, the female moths emerged from larvae reared on diets A and B lived (1.11 and 1.49 days, respectively) longer than female moths reared on the standard diet. On the other hand, no significant differences were recorded among all the tested diets for male moths (Fig. 2).

In addition, the results demonstrated that no significant differences were observed in the sex ratio parameter for insects reared on all tested diets (Table 5). The results shown in Table 5 demonstrate that, the average number of eggs laid was not significantly affected by diets. However, the number of eggs laid by females fed diet (A) was higher (1434.6±762.81 eggs) than those fed on the standard diet and on diet (B) (1127.1±804.09 and 1402.8±993.27 eggs, respectively). Percentage of 61.27±14.12, 72.28±14.95 and 67.65±16.24 % of egg masses were viable for the standard diet, diet (A) and diet (B), respectively (Table 5).

A more satisfactory larval and pupal growth and development were noted on diets (A) and (B) as evidenced by tested larval and pupal growth indices of 7.08 and 11.27, respectively and a total developmental index of 4.37 for diet (A), which were lower comparing with the standard diet (6.8, 10.52 and 4.3, respectively) and close to 6.91, 11.64 and 4.38 observed on diet (B) (Table 6).

Table 5: Sex ratio (%), fecundity (no. of eggs per ♀) and percentage of fertility of S. littoralis moths obtained from larvae reared on the standard and two modified diets

<table>
<thead>
<tr>
<th>Tested diet</th>
<th>Sex ratio (♀ %)</th>
<th>Fecundity (no. of eggs per ♀)</th>
<th>Fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet</td>
<td>48.72±13.57 a</td>
<td>1127.1±804.09 a</td>
<td>61.27±14.12 b</td>
</tr>
<tr>
<td>Diet A</td>
<td>47.20±11.40 a</td>
<td>1434.6±762.81 a</td>
<td>72.28±14.95 a</td>
</tr>
<tr>
<td>Diet B</td>
<td>48.42±12.92 a</td>
<td>1402.8±993.27 a</td>
<td>67.65±16.24 ab</td>
</tr>
</tbody>
</table>

Means followed by identical letters are not significantly different for comparisons between treatments within each column (P <0.05).

Table 6: Larval and pupal growth and total developmental indices of S. littoralis reared on the standard and two modified diets.

<table>
<thead>
<tr>
<th>Tested diet</th>
<th>Larval growth index</th>
<th>Pupal growth index</th>
<th>Total developmental index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet</td>
<td>6.795</td>
<td>10.516</td>
<td>4.302</td>
</tr>
<tr>
<td>Diet A</td>
<td>7.076</td>
<td>11.271</td>
<td>4.368</td>
</tr>
<tr>
<td>Diet B</td>
<td>6.909</td>
<td>11.635</td>
<td>4.382</td>
</tr>
</tbody>
</table>

Table 7: Cost comparison of the composition of the standard and modified diets used for rearing Spodoptera littoralis larvae

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Standard diet</th>
<th>Diet A</th>
<th>Diet B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haricot beans (gm.)</td>
<td>1000</td>
<td>7.50</td>
<td>1000</td>
</tr>
<tr>
<td>Agar agar (gm.)</td>
<td>60</td>
<td>39.60</td>
<td>20</td>
</tr>
<tr>
<td>Starch (gm.)</td>
<td>0</td>
<td>0</td>
<td>150</td>
</tr>
<tr>
<td>Dried Brewer's yeast (gm.)</td>
<td>150</td>
<td>0.90</td>
<td>150</td>
</tr>
<tr>
<td>Ascorbic acid (gm.)</td>
<td>15</td>
<td>2.40</td>
<td>15</td>
</tr>
<tr>
<td>Methyl p- hydroxy benzoate (gm.)</td>
<td>9.5</td>
<td>3.00</td>
<td>9.5</td>
</tr>
<tr>
<td>Sorbic acid (gm.)</td>
<td>5</td>
<td>0.90</td>
<td>5</td>
</tr>
<tr>
<td>Formaldehyde 40% (ml.)</td>
<td>10</td>
<td>2.30</td>
<td>10</td>
</tr>
<tr>
<td>Distilled water (ml.)</td>
<td>2000</td>
<td>---</td>
<td>2000</td>
</tr>
</tbody>
</table>

Total cost of Ingredients (£) | --- | 56.60 | 30.80 | 37.40 |
To determine the cost impact of modified diets the total cost of ingredients for each diet was calculated which were 56.60, 30.80 and 37.40 £ / 5Kg cooked media for the standard diet, diet (A) and diet (B), respectively (Table 7). 

Numerous diets for Lepidoptera rearing have been already modified/simplified [15, 30, 31, 32]. Most of the diet developed support good insect growth but have been ruled out as potential diets for mass rearing because of their high cost. Worldwide, it’s evident that agar is the most expensive ingredient of insect rearing diet (nearly 70% for the standard diet) and consequently its substitutions would have the greatest effect in diet cost. So much effort has been expended in attempting to evaluate the agar substitutions. In present work, the females fecundity which was fed on diet (A) was higher (1434) than those fed standard diet (1127), which lasted 7.7 and 6.6 days, respectively. Similarly a correlation between the female longevity and the number of eggs laid is also reported by Abbasi et al. [25]. The maximum number of eggs lay by one female (2346) was recorded by Mabrouk et al. [19] for S. littoralis reared on agar-based diet, while El-Awady et al. [20] reported a fecundity of (1000 eggs) for the same diet and the same pest. Also, Gupta et al. [14] reported total developmental index of Spodoptera litura (2.84), which is below the result 4.38 recorded in the present study. The modified diets in this work by substituting the agar amounts partially with starch reduced the cost of ingredient by 45.6% and 33.3% per one litter of diets (A) and (B) respectively. This results agree with that obtained by Mabrouk et al. [19] they found that, when substituted 50% of beans quantity with maize this reduced the expenses per tray of diet by 46.2%, while Abbasi et al. [25] reduced the cost for tapioca-based diet by 2.13 fold.

In conclusion, the present study showed starch (cornflower) can be used as a gelling agent in artificial diet for S. littoralis partial mass rearing. From the cost estimate point of view, diet A proved to be the most suitable and economic. This diet may also be used as a semi-synthetic diet for rearing several other economically important Lepidopterous.

REFERENCES


