An Agar-Free Insect Rearing Artifical Diet: A New Approach for the Low Cost Mass Rearing of The Egyptian Cotton Leafworm, *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae)

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Abstract: A new formula for insect rearing artificial diets without using any of their conventional gelling agents was provided in the present work, for the first time. Larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) were successfully mass-reared, for nine consecutive generations on an agar-free artificial diet. This suggested medium is based on yellow lentils, *Lens culinaris* and rice, *Oryza sativa* as the basic constituents. An accurately amounted ratio between these two highly nutritive constituents produces, when cooked, a firm gel with desirable physical properties for insect-rearing artificial media. Through 9 consecutive generations, the *S. littoralis* biological results of the developmental durations, pupation %, pupal weights, sex ratio, adult emergency, longevity, fecundity and egg fertility, were either comparable or superior to those recorded for both the reference natural diet, the castor bean leaves *Ricinus communis L*. and the previously reported results on agar-or alginate-based media for rearing the present insect On this diet, *S. littoralis* larvae have been reared for 9 generations with no noticeable decline in their yields of pupae or moths and fecundity or hatchability. The estimated cost of the suggested artificial diet ingredients for rearing 1000 newly-hatched larvae until pupation, was about 6.2L.E. (ca., $1.0). On the other hand, the cost of such an agar-free diet was found to be reduced by 60 to 73% when compared to agar-based diets developed by other authors.

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1. Introduction

The mass-rearing of insects under controlled conditions represents an important tool for insect pest control. The successful insect culturing in the laboratory is necessary for efficient and productive research on virtually every aspect of insect biology (Knipling, 1966). One important consideration for the mass-rearing of insects is the diet provided to the larvae and adults. Rearing insects on their natural host may not be feasible for a number of reasons including seasonal availability, excessive costs, and variable quality. Therefore, mass-reared insects tend to be provided with artificial diets that bear little resemblance to their natural host or food source but nonetheless permit satisfactory growth and development of the mass-reared insects (Vanderzant, 1966).

Since the first attempt by Bottger (1942) to rear a phytophagous insect, the European corn borer *Ostrinia nubilalis* (Hübner), on an artificial diet, a number of insects have been reared on diets (Shorey and Hale, 1965; Smith, 1966; Dimetry, 1970; Klein *et al*., 1981; Navon, 1985; Singh and Moore, 1985; Alfazairy, 2011). The Egyptian cotton leafworm *Spodoptera. littoralis* (Boisd.) is a key pest of cotton that has a worldwide economic impact. It has been mass-reared on artificial diets in order to establish and maintain a laboratory continuous culture for research on control measures or the effectiveness of its biological control agents (Dimetry, 1970; El-Minshawy and Zeid, 1972; Khalifa *et al*., 1973; Hegazi *et al.*, 1977; Garcia *et al*., 1984; Navon, 1985; Simmonds *et al*., 1992; Anderson *et al*., 1995; Mabrouk *et al*., 2001; Amin *et al*., 2006; Khodaverdi *et al*., 2010). These diets have included an array of components such as soybeans, beans, peas, maize grains, wheat or wheat germ, potato, leaf-meals of alfalfa, corn, cotton or castor beans, milk powder, yeast powder, vitamins, and ascorbic acid .

Concerning the preparing of artificial diets for rearing and culturing the insects, the available literature reveals that the most significant cost item is agar which comprises 70% (Mabrouk *et al*., 2001) or 73% (Hegazi *et al*., 1977) of the total price of the diet. Due to the high cost of agar-based diets for *S. littoralis*, there is a necessity for the substitution or manipulation of new diet ingredients and the development of more cost-effective rearing techniques (Raulston and Lingren, 1969; Raulston and Shaver, 1970). For example, Raulston and Shaver (1970) investigated the possibility of reducing the amount of agar needed, in the casein-wheat germ diet used for rearing tobacco budworm, *Heliothis* *virescens* (F.) and the bollworm, *Heliothis zea* (Boddie), by adding low cost corn cob grits. It was found that the cost of the subject diet was reduced 25% when the corn cob grits were used (Raulston and Shaver, 1970). Navon and Keren (1980) found that an alginate gel system, as an agar substitute, was useful for rearing *S.littoralis*. The use of this gelling agent in insect diets was less costly than agar, and was firstly introduced by Moore and Navon (1969). In a further attempt to develop a practical-low cost diet for use in the mass-rearing environment, the present study was carried out aiming to develop a practical, low cost an agar-free diet for mass-rearing of *S. littoralis*.

2. Material and Methods

Diet composition and preparation. The suggested diet contain 180g yellow lentils, *Lens culinaris*, 25g rice, *Oryza sativa*, 18.5g brewer's yeast powder, 3g ascorbic acid, 4g sorbic acid, 2.5g sodium benzoate, 1mL formalin (37-40%) and 575mL tap water. In a pot, the yellow lentils, rice, and water were cooked for nearly 9-12 min. Ascorbic acid and yeast were added after the pot had cooled. The mix was then blended in a 1¼ litre electric blendor. Formalin, followed by the remaining constituents, was added and thoroughly blended. The diet was, then, dispensed into 9-11 clear plastic rearing containers (15 × 10 × 7cm).

On dispensing the diet into the rearing containers, the poured diet in each container (ca., 3-tablespoon) needs to be gently agitated to distribute the diet allover the bottom of the rearing container, as well as to achieve the desired diet thickness. The diet-filled containers were left to solidify and cool before each container was covered by its plastic lid. All rearing containers were then stored, at 4-7ºC, until use.

Larval rearing. The egg patches or newly hatched larvae of *S. littoralis* were originated from a collection of 4^{th}-to 6^{th}-instar larvae from a sugar beet field near Alexandria, Egypt, in 2009. For routine rearing, 750-1000 newly hatched larvae, per rearing container, were scattered over the diet surface, or upon hatching, egg-patches were glued onto the inner sides of other rearing containers. Each rearing container, infested with larvae or egg-patches, was tightly covered with a cotton cloth by means of a rubber band; then placed inverted for 7-9 days. Such an upside-down position results in the larvae of the first three instars will move toward the diet and feed normally, as well as the larval feces will accumulate on the cloth cover of the rearing container, which provides an easy means of maintaining sanitary conditions within the rearing container (Fig. 1). Larvae were transferred to fresh diet three times, on the 6^{th} day (750-1000 larvae per container), 9^{th} day (350 larvae per container), and 12^{th} day (100 larvae per container) during the larval development.

For the pupation, full-grown larvae tended to either tunnel through the diet to prepupate and then pupate, or pupate on the surface of the diet. Pupae were weighed and sexually discriminated at the beginning of the stage. Pupae were then put into containers with a layer of sawdust until moth emergence. Newly emerged moths were placed for feeding and oviposition in large jars or containers (sex ratio is: 1 ♂: 1♀). The latter were lined with the A4 writing paper and provided with pieces of the writing paper folded into corrugations (Fig. 1) to be used as oviposition sites and resting places for moths. Any container or jar was tightly covered with a cotton cloth. Moths were fed a 10% sucrose solution. Oviposition was observed and adult longevity was recorded.

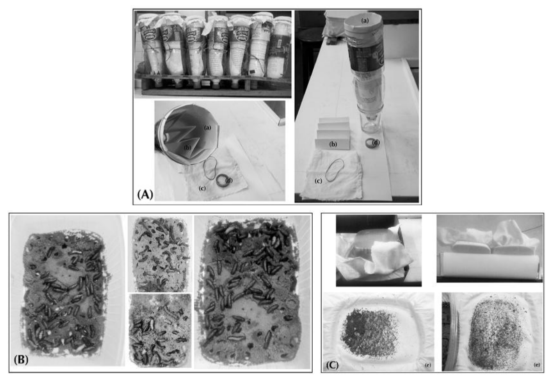

Fig. 1. Technique and facilities required for *Spodoptera littoralis* mass rearing. (A) Rack holding units for a single pair mating and oviposition; (a) plastic bottle (volume: 1.5L) lined with an A4 writing paper, (b) resting and egg-laying corrugated paper, (c) cotton cloth cover and rubber band, (d) bottle screw cap provided with cotton wick saturated with sugary solution for moth-feeding. (B) Larval rearing container showing pupation. (C) Upside-down position of the larval rearing containers, at their first three larval instars to get rid of feces easily from the container cotton cloth cover (e).

Evaluation of some biological parameters. From the previous larval rearing routine on the subject diet, one hundred newly-hatched larvae were used to measure the larval and pupal durations, pupal weight, pupation (%), adult sex ratio, fecundity and longevity of *S. littoralis*. During the larval developmental period, larvae were transferred to fresh diet three times, on the 6^{th}-, 9^{th}-and 12^{th}-day. Developed pupae were weighed, sexed, and counted. For a comparison purpose, a parallel group of larvae had been fed on castor bean leaves and manipulated as done in the tested group.

Fecundity and longevity of *S. littoralis* moths developed from larvae reared on both the artificial diet or the castor bean leaves were compared. One pair of moths taken from the artificial diet or from the reference diet was placed in a plastic bottle (volume: 1.5L). This bottle served as a mating and oviposition unit for a single pair of moths. As shown in Figure 1, the bottle was lined with A4 writing paper, and its bottom was cut and replaced by a tightly fixed cotton cloth cover. In addition to the paper lining, there was a corrugated paper that serves as a resting place for the paired moths. Both papers served as oviposition sites. The bottle screw-cap was provided with a small cotton wick saturated with a 10% sugar solution that served as food for the moths. A wooden rack was designed to hold the oviposition units upright with the screw-cap down and the cotton cloth cover end up (Fig. 1).

Both rearing procedures were conducted under the same laboratory conditions (19-31ºC, 74-92% RH, and 13:11 hours L:D photoperiod).

Statistical analysis of data. Means of each pair of results from the agar-free diet and the reference diet of each generation were compared by using t-tests at the 5% level of probability.

3. Results and Discussion

As it is known, nearly all the artificial diets used for the laboratory rearing of phytophagous insects are agar-based ones. Agar as a gelling agent is one of the most expensive components of the diet (Raulston and Shaver, 1970). Other less costly gelling agents, such as cellulose (Vanderzant, 1966); calcium-alginate and citric acid (Navon and Keren, 1980) have been suggested for the artificial diets of many insects. Therefore, the present new approach for using a much less expensive rearing diet focuses on the possibility of dispensing with agar without changing the physical properties of the diet. Preliminary tests (data not shown) revealed that 180g of yellow lentils, *L.culinaris* and 25g rice could be used successfully as diet nutritive components and gelling agents without affecting the diet physical properties for avoiding a probable negative effect on the larval growth and pupation.

Such a new approach in gelling technique of the artificial diets for rearing phytophagous insects is based on a well known physical property (i.e., solidification) of lentils or rice when cooked with a certain amount of water for a certain time. Therefore, it was very important to estimate, firstly, the amount of lentils, rice, and water to be formulated together; secondly, the time required for cooking. The 180g of lentils, 25g of rice, and 575ml water (cooked for 9 min) were the most suitable formulation in order to achieve the desired firm-gel texture and consistency of the medium. Based on both the nutritional value of lentils and rice, the present diet seemed to be nutritionally suitable, and characterized by proper physical properties that are desirable in an insect artificial diet. The diet has a quite, hard or firm, not sticky, and spongy consistency. In this concern, Moore and Navon (1974) reported that insects normally chewing plant tissues can be expected to adapt to an artificial diet which is nutritionally adequate and has a firm, non-sticky surface and a relatively hard with a spongy infra-structure. The results shown in Tables 1−4 and Figure 1 reflect to how extent the present artificial diet was successful and satisfactory for *S. littoralis* laboratory mass-production.

Moore and Navon (1974) reported that in most of the artificial diets for chewing insects, the requirement for firmness has been met by agar. Agar is a relatively inert binding agent; therefore it produces a stable gel. In the present diet, however, an auto-gelling technique was achieved by means of the diet nutritive components themselves, yellow lentils and rice, not by adding agar or other expensive gelling agents. That makes this diet too cheap and practical, as well as so simple to prepare. Hence, as compared with agar-based diets or alginate-based diets, the present agar-free diet needs markedly less time to prepare where procedures of heat-dissolving of agar or preparing an alginate gel system are not needed. Navon and Keren (1980) reported that, in diets that based on an alginate gel system as an agar substitute, a firm gelled diet was developed within 20-30 minutes. Also, this gelling technique involves a certain time-consuming procedures.

The other nutrients of the present agar-free diet were dried yeast and ascorbic acid. The diet anti-contaminants were sodium benzoate, sorbic acid, and formalin. To evaluate the use of such an agar-free diet as a satisfactory diet for *S. littoralis*-mass rearing, some biological parameters were studied and compared, for nine successive generations, with the corresponding data of rearing it on castor been leaves, as a reference diet.

Larval duration. For nine consecutive generations, under laboratory conditions cited in Table 1, the larval developmental period was, in general, insignificantly shorter as calculated in 0.3−3.5 days (14.8 ± 0.1 to 25.7 ± 0.9 days) on the subject agar-free artificial diet than on the reference diet, (14.9 ± 0.3 to 27.5 ± 0.5 days). The insignificantly (p=0.05) shortened larval developmental period may be attributed to the food constituents (Engelmann, 1970). In this concern, Abdel-Fattah *et al*. (1977) found that the most favourable host plants which shortened the *S. littoralis* larval period had contained more carbohydrates, total nitrogen and microelements. One of these host plants was the castor bean leaves that contain 23.57mg carbohydrates, 5.18g total nitrogen per 100g, as well as calcium, magnesium and potassium. On the other hand, the USDA nutrient database revealed that the corresponding nutritional value per 100g of the yellow lentils, the main nutritive and gelling constituent in the present suggested artificial diet, seems to be nearly higher by 3-fold and 5-fold for, respectively, carbohydrates (60g/100g) and protein (26g/100g). Also, the yellow lentils, nutritionally, contains high percentages of vitamin B_1, and B_9, calcium, magnesium, potassium, iron, phosphorus, sodium, and zinc. Moreover, the yellow lentils contain 2g sugars, 31g dietary fiber, and 1g fat/100g. Rice as another nutritive and gelling component in the present artificial diet has more carbohydrates (80g/100g) and less protein (7.13g/100g), sugars (0.12g/100g), dietary fiber (1.3g/100g), and fat (0.66g/100g) than the yellow lentils. Rice contains, also, microelements as the yellow lentils, but with different percentages in addition to vitamin B_1, B_2, B_3, B_4, B_5, and B_6. Therefore, the present an agar-free artificial diet appears to be nutritionally superior to the reference diet, castor bean leaves. However, reported results of the *S. littoralis* larval developmental period on both the agar-based artificial diets (Salleh, 1976; Hegazi *et al*., 1977; Mabrouk *et al*., 2001) and the alginate-based diets (Moore and Navon, 1969) may consider the subject diet as a new approach in the artificial rearing of insects.

Pupation and pupal weight. Data presented in Tables 1 and 2 indicate that, through nine consecutive generations of the cotton leafworm, *S. littoralis* on both diets, the percentages of pupation on castor leaves (51-100%) were, in general, a little bit lower than those on the artificial diet (63-100%). Additionally, the pupal duration for larvae reared on the reference diet was prolonged by about 1-, 2-, 3-, or 5- day as compared with the corresponding data of the artificial diet (Table 1).

Table 1. Developmental durations (Mean±SE) of *Spodoptera littoralis* reared, for 9 consecutive generations, on an agar-free artificial diet and on a reference diet, castor bean leaves

Lab. conditions and generation (G)		Duration in days			
		Artificial diet		Reference diet	
		Larval stage	Pupal stage	Larval stage	Pupal stage
		Mean * ± SE (Range)	Mean * ± SE (Range)	Mean * ± SE (Range)	Mean * ± SE (Range)
G_1	23.1 ±1.3ºC; 82.0±6.6%RH	24.3±0.5 (23-27)	13.8±0.6 (13-19)	24.6±0.5 (21-26)	16.6±0.6 (14-19)
G_2	21.3±0.9ºC; 81.4±1.3%RH	24.0±0.3 (23-25)	12.7±0.4 (12-15)	27.5±0.5 (27-32)	13.6±0.3 (13-16)
G_3	25.3±0.8ºC; 81.7±2.8%RH	20.9±0.8 (17-24)	12.4±0.2 (12-13)	20.6±0.8 (17-23)	12.5±0.3 (12-15)
G_4	27.8±1.3ºC; 86.4±3.5%RH	15.5±0.3 (15-17)	5.3±0.2 (5-6)	14.9±0.3 (13-15)	10.2±0.2 (10-12)
G_5	29.9±0.8ºC; 87.8±2.2%RH	15.6±0.7 (14-19)	10.5±0.2 (10-12)	16.2±0.3 (15-17)	10.6±0.4 (9-12)
G_6	30.7±0.5ºC; 85.5±2.0%RH	17.7±0.3 (17-19)	11.0±0.4 (10-13)	17.1±0.5 (15-19)	10.9±0.5 (8-12)
G_7	30.3±0.8ºC; 83.9±2.0%RH	14.8±0.1 (14-15)	11.5±0.6 (10-15)	15.2±0.7 (13-21)	11.4±0.5 (9-13)
G_8	26.8±1.6ºC; 80.5±3.7%RH	20.4±0.5 (19-23)	13.0±0.5 (12-16)	20.1±0.8 (17-23)	12.5±0.3 (10-13)
G_9	19.0±1.2ºC; 79.3±3.3%RH	25.7±0.9 (24-32)	15.2±1.3 (14-19)	27.1±1.2 (22-32)	16.8±0.7 (15-21)

* Results are not significantly different at the 5% level of probability by t-test ($p = 0.05$).

Based on the statistical analysis, those pupae developed from larvae reared on both diets revealed an insignificant difference. However, the pupae obtained from the agar-free diet were, in general, slightly heavier than those produced from the reference diet, castor bean leaves. In general, the pupae recovered from the artificial diet were heavier by 14-103mg than those obtained from the reference diet (for details, see data assorted in Table 2).

Table 2. Pupation (%) and pupal weight (Mean±SE) of *Spodoptera littoralis* for 9 consecutive generations reared on the artificial diet and the natural reference diet.

Generation (G)	Pupation (%)		Pupal weight (mg)	
	Artificial diet	Reference diet	Artificial diet	Reference diet
			Mean * ± SE (Range)	Mean * ± SE (Range)
G_1	63	63	230±16 (160-330)	242±18 (150-350)
G_2	74	51	242±15 (210-350)	221±17 (170-320)
G_3	73	59	242±16 (170-330)	257±14 (180-330)
G_4	97	100	257±13 (180-330)	224±14 (160-300)
G_5	100	82	224±9 (180-280)	210±9 (170-260)
G_6	87	66	239±8 (190-280)	240±17 (190-350)
G_7	100	86	253±9 (190-290)	237±10 (190-290)
G_8	100	100	284±8 (220-310)	236±21 (170-360)
G_9	74	68	347±6 (320-380)	244±13 (200-320)

* Results are not significantly different at the 5% level of probability by t-test (p = 0.05).

As a result of the continuous rearing of S. littoralis on the present suggested agar-free diet for nine consecutive generations, there was an obvious increase by 9-117mg in the average weights of pupae recovered from the larvae reared on this artificial diet as compared to the corresponding average for pupae of the 1st generation (Table 2). Hence, no adverse effects were observed from the continuous rearing on the subject agar-free diet. Also, the observed successful pupation and the large yield of pupae recovered from this diet may serve as an indication of the satisfactory physical and nutritional properties of this new diet. Figure 1 shows a successful pupation either on the surface of the diet or in tunnels made by the larvae upon pupation. An overview on referenced data for S.littoralis larvae reared on either certain agar-based artificial diets, which modified from that of Shorey and Hale (1965), or alginate-based diets (Moore and Navon, 1969; Navon and Keren, 1980) may prove to how extent the present new diet is satisfactory for economic mass-production of the Egyptian cotton leafworm, S. littoralis.

Adult performance. The yield of the agar-free artificial diet-reared moths was, in general, slightly higher than that of the natural reference diet (Table 3). The percentages of adult emergence of both diets were comparable and ranged between 74 and 100% for the artificial diet, and between 54 and 100% for the reference diet. The moth emergence was sometimes quite similar on both diets, and other whiles considerably higher or slightly higher than that of the reference diet (Table 3).

Oviposition records for individual female moths recovered from both test diets were statistically compared by using t-test at the 5% level of probability. Egg-production by the diet-emerged females, through 9 consecutive generations, was not significantly different from that of the castor leaves- emerged females. The average yield of eggs per female was statistically comparable on both diets; fecundity of the artificial diet- emerged moths was, in general, 38.9‒839.5 eggs more than that of castor leaves-developed moths (Table 4).

Based on the statistical analysis by adopting t-test at the 5% level of probability, the average numbers of hatched eggs from the test agar-free artificial diet and the reference diet were also not significantly different. On the non-agar diet, the percentages of eggs hatched or egg fertility, for 9 successive generations, were sometimes 1.8 to 8.3% higher; or 1.3 to 2.2% lower on the artificial diet than on the natural diet (Table 4).

Table 3. Adult emergence (%) and longevity (Mean±SE) of *Spodoptera littoralis* after rearing larvae on both the artificial diet and the natural reference diet through 9 consecutive generations

Generation (G)	Adult emergence (%)		Adult longevity (days)	
	Artificial diet	Reference diet	Artificial diet	Reference diet
			Mean * ± SE (Range)	Mean * ± SE (Range)
G_1	93.7	63.5	8.2±0.9 (4-11)	7.8±0.7 (5-11)
G_2	74.3	76.5	6.5±0.8 (4-12)	6.3±1.0 (3-12)
G_3	82.2	54.2	7.1±0.7 (4-12)	6.4±0.8 (4-12)
G_4	95.9	100	5.8±0.5 (4-7)	6.0±0.4 (4-8)
G_5	100	80.5	8.2±0.4 (7-12)	5.8±0.6 (2-8)
G_6	100	83.3	5.4±0.3 (4-6)	3.1±0.2 (2-4)
G_7	100	80.2	9.5±1.3 (5-17)	10.2±1.6 (3-18)
G_8	100	60.0	8.5±0.9 (4-11)	7.2±0.4 (4-9)
G_9	74.3	73.5	8.5±0.8 (5-12)	8.2±0.6 (6-11)

* Results are not significantly different at the 5% level of probability by t-test (p = 0.05).

Table 4. Female fecundity (mean number of eggs/female) and egg fertility (hatching %) of *Spodoptera littoralis* after rearing larvae on both the artificial diet and the natural reference diet through 9 consecutive generations

Lab. conditions and generation (G)		Fecundity		Egg-fertility	
		Artificial diet	Reference diet	Artificial diet	Reference diet
		Mean±SE (Range)	Mean±SE (Range)	Mean±SE (Range %)	Mean±SE (Range %)
G_1	23.1 ±1.3ºC; 82.0±6.6%RH	1008.5±95.5 (774-1272)	800.7±99.6 (419-1593)	94.5±1.8 (85.4-100)	94.6±2.2 (83.5-100)
G_2	21.3±0.9ºC; 81.4±1.3%RH	986±106.3 (660-1640)	947.1±190.9 (460-2512)	94.9±1.5 (86.2-100)	97.1±1.3 (86.7-100)
G_3	25.3±0.8ºC; 81.7±2.8%RH	1325±152.2 (829-2245)	520.4±26.2 (420-642)	94.5±1.1 (90.1-100)	92.7±3.3 (70.3-100)
G_4	27.8±1.3ºC; 86.4±3.5%RH	1899±72.1 (1588-2312)	1624.3±185.1 (915-2763)	100	100
G_5	29.9±0.8ºC; 87.8±2.2%RH	2059.8±57.8 (1729-2310)	1220.3±168.4 (412-2118)	100	100
G_6	30.7±0.5ºC; 85.5±2.0%RH	2021.5±79.4 (1644-2416)	2101.7±47.0 (1870-2311)	100	100
G_7	30.3±0.8ºC; 83.9±2.0%RH	1455.2±128.4 (936-2359)	2060.3±22.6 (1980-2173)	95.1±1.3 (88.9-100)	96.5±1.1 (89.2-100)
G_8	26.8±1.6ºC; 80.5±3.7%RH	1905.3±137.6 (990-2205)	1172.1±128.1 (406-1640)	95.6±0.9 (92.6-100)	93.8±3.6 (65.4-100)
G_9	19.0±1.2ºC; 79.3±3.3%RH	1010.7±101.6 (558-1623)	897.6±95.4 (526-1533)	59.4±5.4 (43-100)	51.1±4.9 (28.7-78)

* Results are not significantly different at the 5% level of probability by t-test (p = 0.05).

It was noteworthy that, upon hatching, *S. littoralis*–egg patches of the 9th generation that were maintained at laboratory conditions of comparatively low temperature (19 ± 1.2ºC) and relative humidity (79.3 ± 3.3%) had recorded an obvious decrease in hatchability on both diets (59.4 and 51.1% for the artificial – and reference – diet, respectively; Table 4). Such a remarkable reduction in egg fertility (ca., 35 – 41% or 42 – 49% on the artificial - or natural – diet, in respect) was attributable to the recorded sharp reduction in temperature by ca., 11 to 12ºC; as compared with the results recorded at ca., 30 to 31ºC where the percent egg hatch was 100%, and about 93 to 97% at ca., 21 – 28ºC, with relative humidity ca., 81 – 88% (Table 4). This is in agreement with previous findings reported by Rockstein (1973) and Navon (1985).

Longevity of adult developed from *S. littoralis* larvae reared, for 9 consecutive generations, on both test diets was not significantly different. For the nine generations, the records of moth longevity of both diets were comparable, but, in general, artificial diet-emerged moths seemed to live, on the average, 0.2 – to 2.4–day more than those of the natural reference diet (Table 3). Also, through the test 9 generations, results indicated that the sex ratio is about 1:1.

An overview on the test adult stage emergence, longevity, fecundity, egg fertility, and sex ratio reveals that the present biological records on the subject agar-free artificial diet were, to a large extent, comparable and recorded similar or superior results to those either obtained on the present natural reference diet, fresh castor bean leaves, or those previously reported by certain investigators on agar-or alginate-based diets (Moore and Navon, 1969, 1971 and 1973; Dimetry, 1970; El-Minshawy and Zeid, 1972; Salleh, 1976; Hegazi *et al*., 1977; Navon and Keren 1980; Amate *et al*., 2000; Mabrouk *et al*., 2001; Khodaverdi *et al*., 2010).

Based on the observed normal growth or development of the Egyptian cotton leafworm, *S. littoralis*, and the satisfactory yields of its pupae and moths, for 9 consecutive generations, and to date, on the present agar-free diet with no noticeable decline in average pupal weights and adult reproductive potential (i.e., fecundity and fertility), as well as adult longevity; also taking into consideration the corresponding data for rearing *S. littoralis* on its natural food (castor bean leaves) and its other previously reported agar-or alginate-based artificial diets, the subject diet seemed to be an acceptable promising artificial mass-rearing diet for the Egyptian cotton leafworm.

Since the present agar-free diet allowed successful growth and development for *S. littoralis*-immature or mature stages with no deformed pupae or adults, that may considerably prove its nutritional adequacy and its desirable physical properties which enhance the insect biological quality.

Undoubtedly, the cost of insect rearing media is an important economic criterion for producing such media. In this study, the estimated cost of the subject artificial diet ingredients for rearing 1000 newly-hatched larvae of *S.littoralis* till their pupation was 6.2L.E. (ca., $1.0). On the one hand, Raulston and Shaver (1970) reported that the cost of a low agar casein-wheat germ diet, for rearing tobacco budworms, by adding low cost corn cob grits was reduced by 25%; on the other hand, the present agar-free diet would reduce the cost of rearing larvae of the Egyptian cotton leafworm, *S.littoralis* by 60 to 73% of the total price of the artificial diet used. As the diet represents one of the most costly components of insect rearing programmes, and is especially important when rearing large numbers of larvae for production of certain bioinsecticides or for release in field trails, therefore, the present estimate reflects the desired low cost which would allow mass-production of insects with minimum costs.

In conclusion, the agar-free artificial diet developed in this study has proved to be satisfactory for mass-rearing the Egyptian cotton leafworm, *S. littoralis* with no noticeable tendency toward a decline of development or reproduction for 9 consecutive generations. This diet costs about 60-73% less than the agar-based diets, and provides a new approach for further investigations of less expensive gelling agents.

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