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Fecundity, egg fertility and longevity of laboratory reared the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelichidiea) under different adult diet regimes

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Abstract:

Reproduction of most insects depends on nutrients accumulated during the larval stage. Recent studies, however, highlight the fundamental importance of adult nutrition. Feeding at the adult stage allows the intake of carbohydrate, lipids and amino acid rich solutions, which may have an effect on the species reproduction and population growth. Fecundity, egg viability and adults longevity data were collected for adult *Pectinophora gossypiella* (Saunders)(Lepidoptera: Gelichidiea) maintained on one of eight adult diets, these are distilled water, which was used as a control (T1), 20% sugar solution (T2), 20% honey solution (T3), 20% sugar solution + 3 drop of food oil (T4), 20% sugar solution + 10% yeast (T5), 20% sugar solution + 5% yeast (T6), 20% sugar solution + 10% yeast + 3 drop of food oil (T7) and 20% sugar solution + 5% yeast + 3 drop of food oil (T8) at two concentrations. The results indicated that moth performance was poor on honey (T3) or 10% yeast (T5) diets, best on oil diet (T4) and moderately on other diets (T2, T6, T7 and T8). Consistently oil diet (T4) enabling the moths to live moderately (13.59 and 13.87 days for female and male, respectively), produce more eggs (118.92 egg/female) and had high reproductive capacity as the percentage of control (165.98%). Also, they relatively inexpensive; thus, it can be considered good diets for maintaining laboratory colonies of these moths. In conclusion, adult's diet can play an essential role in egg production and in sustaining longevity of females.

Introduction

The cotton bollworm moth, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelichidiea) is considered one of the most important pest infested cotton crop. This pest is very active fliers and larvae mostly remain inside squares, flowers and bolls and cause severe damage (De Melo *et al*., 2012). Adult of lpidopterous insects depends on larval derived nutrients for reproduction (Geister *et al*., 2008). Nutritional requirements may vary among adults of different species. Among numerous nutrients (notably, proteins, carbohydrates, lipids, vitamins and mineral elements) discussed by House (1965), it seems that carbohydrate is the most important ingredient in the adult's diet affecting egg production and survival of many lepidopterous adult. In addition, amino acids derived from the adult diet play a role in some species, because amino acids can be incorporated into eggs (Boggs, 1997 and Mevi-Schutz and Erhardt, 2005). Whilst, role of lipids in adult's diet were largely neglected (Beenakkers, 1985). Authors use various types of diets to feed moths in the laboratory (a single diet or a combination of diets, is often used to enhance moth performance. Two of the more commonly used diets for moths are honey solution or a sugar solution) (Adkisson, 1961; Paul *et al*., 1987; Muralimohan *et al*., 2009 and Jothi *et al*., 2016)

The purpose of this study was to select a good adult's diet for use in researches, as well as for use by insect rearing unit of pink bollworm. Insect feeding which results in good egg production and longevity are two important considerations of a good diet. Adults were fed eight diets to determine the effects of water, sugar, honey, food oil and yeast on reproduction maintenance.

Materials and methods

1. Insect: In order to assess the reproductive performance, *P. gossypiella* was obtained from a laboratory colony maintained at the Bollworms Research Department Laboratory, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. Newly eclosed adults (<24 h old) were confined in the glass chimney cage. The top and bottom of each cage were covered with screening mesh kept in position by rubber bands for stimulating egg-laying response in females. Five pairs of moths were set up for each treatment with three replicates. Eggs laid by female on the piece of paper placed over and under the cage in open Petri dishes through the screening mesh were collected every 48 hours. Moths and any resulting eggs were held at 27 ± 1°C and 75 ± 5% relative humidity (RH). Pairs were provided with the different adult diets inside the glass chimney with a piece of cotton wool previously soaked in each diet. Solution was suspended to be renewed every 48 hours for moths feeding.

2.Diets: Adults were fed eight diets to determine the effects of water, sugar, honey, food oil (as source of lipids) and yeast (as source of protein and amino acids) on reproduction maintenance:

2.1. Distilled water, which was used as a control (T1)
2.2. 20% sugar solution (T2)
2.3. 20% honey solution (T3)
2.4. 20% sugar solution + 3 drop of food oil (T4)
2.5. 20% sugar solution + 10% yeast (T5)
2.6. 20% sugar solution + 5% yeast (T6)
2.7. 20% sugar solution + 10% yeast + 3 drop of food oil (T7)
2.8. 20% sugar solution + 5% yeast + 3 drop of food oil (T8)

The following parameters were analyzed: fecundity, egg viability and adult's longevity. Fecundity was calculated from the mean number of eggs laid during the entire lifecycle of the females, while egg viability was assessed from the mean percentage of hatched larvae in the treatments. The following equation was used for calculating the percent of fecundity and percent of egg viability:

$$\%\text{Control of fecundity or egg viability} = \frac{\text{C - T}}{\text{C}} \times 100$$

Where; C: The estimated parameter in check
T: The same parameter in treatment

On the other hand, percent of reproduction control (% sterility) was calculated according to the equation of Toppozada *et al*. (1966) as follows:

$$\% \text{Sterility} = 100 - \left(\frac{a \times b}{A \times B} \times 100 \right)$$

Where; a: Number of eggs laid/female in treatment

b: %Hatch in treatment

A: Number of eggs laid/female in control

B: %Hatch in control

3. Statistical analyses: Analysis of variance (ANOVA) was conducted on all data using Costat computer program software. Means were compared by Duncan's multiple range test (Duncan, 1955).

Results and discussion

1. Egg production and fecundity percent:

The food source offered had an apperent effect on the butterflies' ability to lay eggs as well as on egg fertility (Table, 1 and Figures, 1 and 2). The number of the average egg ranged between 55.12 to 118.92 egg/female. Adult feeding just water (T1; control) had the lowest number of laying egg (55.12 egg/female). When feeding on honey solution (T3), the number of eggs laying increased poorly by 3.86% to reach 57.75 egg/female. The diet containing sugar (T2) results in significantly more eggs than the non-sugur diet (91.48 egg/female) by 65.97%. This indicate that sugars aid to maintain egg development during adult ageing and the kind of sugar had an effect.

Yeast had a slightly effect on fecundity when offered in sucrose solution at 10% concentration (T5; 72.75 egg/female) compared with females fed with water only. The percentage of increasing in egg production (% fecundity) was 31.98%. But at 10% concentration with oil (T7) it increased fecundity significantly (103.65 egg/female) by 88.04%. Also, it increased fecundity significantly when offered in a sucrose solution at 5% concentration alone (T6; 92.73 egg/female) or with adding oil (T8; 104.0 egg/female). It was higher than control (females fed with water only) by 68.23 and 88.67% for T6 and T8, respectively.

Total lifetime egg production by butterflies fed diets containing mixture of sugar & oil (T4; 118.92 egg/female) is significantly greater than by butterflies fed other diets. The percentage of increasing in egg production (% fecundity) was 115.75%. This indicate that the existence of oil in the feeding diet has a notable effect (T4, T7 and T8).

2. Hatchability of deposited eggs and viability percent:

The average of egg hatchability percent reached 60.78 % in the case of control (T1) pink bollworm (Table, 1). It was found no significant difference in the probability of being fertile in all treatment vs. conrol except T2 and T6 which differed significantly from control and record 80.44 and 78.25 % of hatchability, respectively, but did not differed significantly from other treatments. However, the calculated percentages of egg viability control were higher than the control by 32.28, 22.23, 23.28, 11.17, 28.74, 8.29 and 10.78%, for T2, T3, T4, T5, T6, T7 and T8, respectively.

3.Reproductive capacity:

It was obvious from the results in the Table (1) that the pattern of fecundity and hatchability exhibited by pink bollworm moths due to the different treatments was reflected in the values obtained for reproductive capacity. Generally, in all treatments, the produced females had high reproductive capacity as the percentage of control, since the control reproduction percentages reached 119.54, 26.95, 165.98, 46.73, 116.59, 103.64 & 109.01% after treatment with T2, T3, T4, T5, T6, T7 and T8, respectively. It could be arranged the tested diet according to its effect on reproductive capacity as follows: T4 > T2 > T6 > T8> T7 > T5 > T3 (Table, 1 and Figure ,2).

Table (1): The effect of different adult diets on reproduction potential and longevity of *Pectinophora gossypiella* moth.

Treatment	Eggs/female ± SE	%Control of fecundity (±)	%Hatchability ± SE	%Control of egg viability (±)	%Control of reproduction (±)	Adult longevity (days) ± SD	
						♀	♂
Water (T1)	55.12^{d} ± 22.28	—	60.78^{b} ± 12.29	—	—	11.5^{b} ± 4.76	10.53^{f} ± 4.12
20% sugar (T2)	91.48^{bc} ± 30.29	65.97	80.44^{a} ± 4.34	32.28	119.54	17.83^{a} ± 6.10	21.4^{a} ± 6.28
Honey (T3)	57.25^{d} ± 11.51	3.86	74.29^{ab} ± 17.59	22.23	26.95	11.38^{b} ± 3.33	10.92^{f} ± 4.39
20% sugar & oil (T4)	118.92^{a} ± 17.20	115.75	74.93^{ab} ± 11.83	23.28	165.98	13.59^{b} ± 1.80	13.87^{de} ± 2.05
20% sugar & 10% Yeast (T5)	72.75^{cd} ± 6.36	31.98	67.57^{ab} ± 12.27	11.17	46.73	17.41^{a} ± 6.04	18.0^{b} ± 5.08
20% sugar & 5% Yeast (T6)	92.73^{bc} ± 8.97	68.23	78.25^{a} ± 17.92	28.74	116.59	18.29^{a} ± 3.43	17.5^{bc} ± 3.09
20% sugar & 10% Yeast +Oil (T7)	103.65^{ab} ± 27.37	88.04	65.82^{ab} ± 18.26	8.29	103.64	12.72^{b} ± 5.14	15.55^{cd} ± 2.81
20% sugar & 5% Yeast +Oil (T8)	104.0^{ab} ± 5.44	88.67	67.33^{ab} ± 9.85	10.78	109.01	16.5^{a} ± 3.63	13.1^{e} ± 2.60
LSD (5%)	**19.44**		**15.20**	—	—	**2.12**	**1.99**

Means followed by the same letter at the same column are not significantly different.

$$\%\text{Control of fecundity or egg viability} = \frac{C - T}{C} \times 100$$

$$\%\text{Control o reprction} = 100 - \left(\frac{a \times b}{A \times B} \times 100 \right)$$

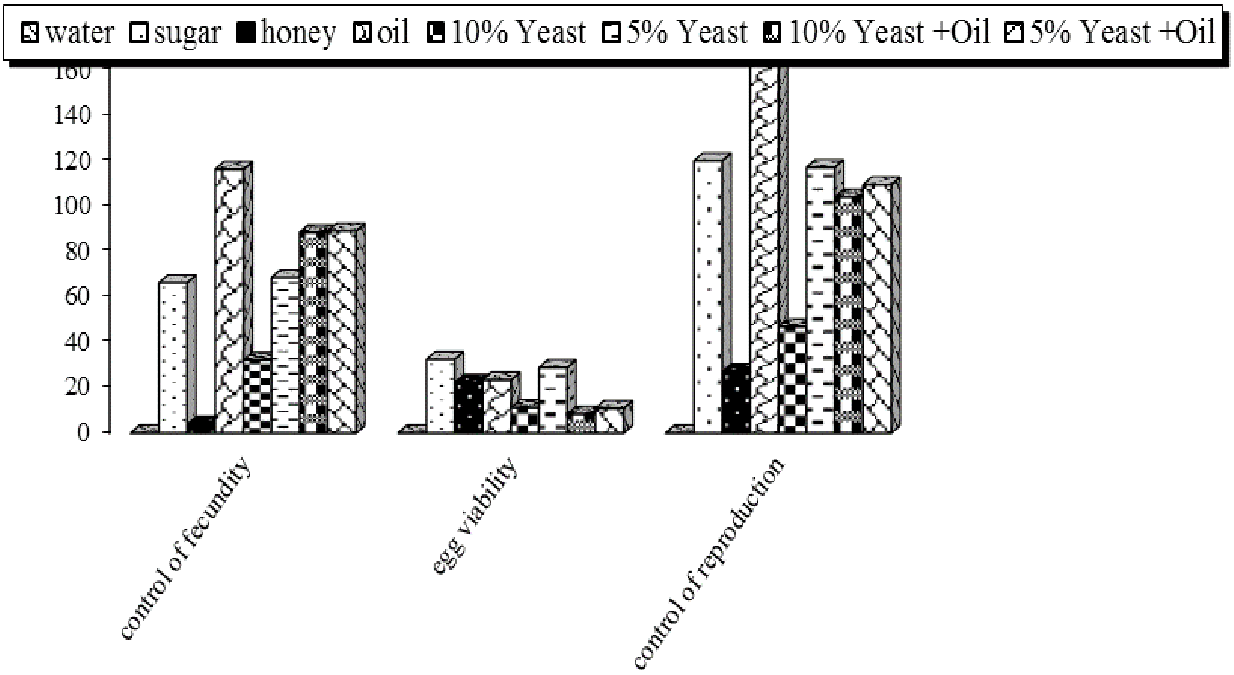

Figure (1): The effect of different adult diets on the fecundity, egg hatchability and longevity of *Pectinophora gossypiella* moth.

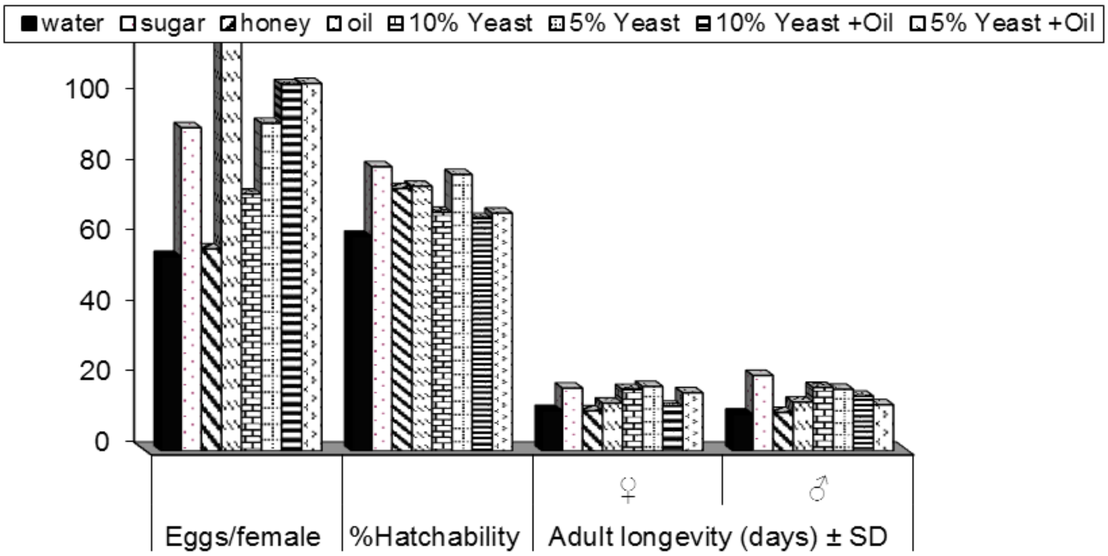

Figure (2): The effect of different adult diets on percent control fecundity, egg viability and reproduction capacity of *Pectinophora gossypiella* moth.

4.Adult longevity:

Adults longevity which feeding on water only was 11.5 & 10.53 days, for femle and male, respectively (Table, 1 and Figure, 1). Sucrose and yeast (5 and 10%) diets were among the diets on which cotton bollworm moth, *P. gossypiella* male and female aduts lived longest (17.83 and 21.4, 17.41 and 18.0 and 18.29 and 17.5 days for sucrose, 5% yeast and 10% yeast, respectively. Conversely, they lived shortest on the honey (11.38 and 10.92 days for female and male, respectively). Oil diets (T4, T7 and T8) was only a moderately good diet for adults (13.59 and 13.87, 12.72 and 15.55 and 16.5 and 13.1 days for female and male, respectively).

Food sources were found to have a significant effect on butterfly fecundity, fertility and longevity of *P. gossypiella* adults. Accumulated information resulted from obove results explain that the existence of oil in the adult diets had the most reproductive capacity. In addition, it had a moderate adult's longevity. It means that reproduction may not only be limited by carbohydrates, but also by deficiencies in other larval-derived substances such as lipids (Behmer and Grebenok, 1998 and Arrese *et al.*, 2001). These results are not in similatry with Bauerfeind *et al.* (2007) who found that lipids, yeast or ethanol added to a sugar solution did not yield a similarly high reproductive output reproduction in the tropical, fruit-feeding butterfly *Bicyclus anynana* (Butler) (Lepidoptera: Nymphalidae) compared to fruit-fed females. Also, Geister et al. (2008) found that in female *B. anynana*, all diet groups (plain sucrose solution, sucrose solution enriched with lipids or yeast) had a substantially lower fecundity and egg hatching success compared to the banana group. Tisdale and Sappington (2001) data indicate that carbohydrates in the adult diet can increase fecundity, fertility and longevity of the beet armyworm, *Spodoptera exigua* (Hübner). Romeis and Wackers (2002) studied the effect of different kind of sugers and found that glucose is the only sugar that has a positive effect on both longevity and fecundity as well as a number of oviposition parameters of *Pieris brassicae*. Also, sugar concentration has been shown to have important effect on most species which have been studied (Leather, 1984). In 1990, Simmons and Lynch collected data about survival and egg production for females of *Spodoptera jrugiperda* (J. E.

Smith), fall armyworm; *Helicoverpa zea* (Boddie), corn earworm *Helicoverpa zea* Boddie and lesser cornstalk borer, *Elasmopalpus lignosellus* Zeller maintained on one of eight adult diets, two honey solution diets, sucrose solution, Gatorade, three beer diets, and water. They found that moth performance was best on honey or sucrose diets. In addition, Jordão *et al*. (2010) found that female fecundity of *Phthorimaea operculella* Zeller was higher in honey-fed females as compared to the water-fed females. In contrast, Euzébio *et al*. (2013) found that the fecundity and longevity of *T. arnobia* (Lepidoptera: Geometridae), adults fed on 15% honey solution did not improve the reproductive capacity and longevity of *Thyrinteina arnobia* (Stoll) females but it favors those of males, which could increase mating probability. This is important because *T. arnobia* males emerge sooner than females, and feeding them could increase their longevity and chances of mating various females.

Therefore, the availability of carbohydrates in the adult diet have a profound impact on the reproductive capacity. While adding yeast (as a source of protein or amino acids) to a soucrose-based diet are generally of low importance and differed according its concentration (also compare Lewis and Wedell, 2007; Molleman *et al.,* 2008 and Bauerfeind and Fischer, 2009), contrasting the findings of Mevi-Schütz and Erhardt (2005) who demonstrated that feeding on amino acid-rich substrates during the adult stage can largely compensate for reduced larval-derived resources in the nectar-feeding map butterfly. As a dietary supplement, yeast has been shown to dramatically increase egg production but to reduce longevity in *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: : Chrysopidae) (Good and Tatar, 2001). The different_results might be due to the fact that the amino acid solutions offered in the various studies differed in their composition and concentration. A second possible explanation could be differences in the nutritional requirements of adult Lepidoptera, depending on the nutrients carried over from the larval stage (Boggs, 1997).

The reproductive potential of lepidopterans is influenced by the insect's lifecycle, nutritional status. Although food resources for reproduction of most insects depend on the nutrients accumulated during the larval stage, many lepidopteran species show feeding habits in the adult stage (Chapman, 1998). Romeis and Wackers (2002) mentioned that many adult Lepidoptera are dependent on carbohydrate-rich solutions such as nectar and honeydew. These food sources can contain a range of carbohydrates as well as low levels of other compounds, including free amino acids, proteins and lipids (Nicolson *et al.*, 2007 and Nepi, 2014). Most of studies have examined only the effect of sugars and amino acids in the diet, and all of them used only a limited range of concentrations. While, few studies so far have attempted to determine the importance of lipids in adult's diet on reproduction.

Carbohydrates represent the primary source of energy for adult Lepidoptera (Boggs, 1987). Also, it has been demonstrated and argued that carbohydrates fed by adults may be an additional food supply that helps the vitellogenin synthesis and egg development, thus increasing fecundity (Tisdale and Sappington, 2001). Since insect eggs are primarily composed of protein and lipid (Engelmann, 1999; Ziegler, 2006 and Karl *et al.*, 2007), we anticipate a high demand for these compounds by ovipositing females. The general importance of lipids and proteins for embryonic and larval development in insects is established (Beenakkers *et al.*, 1985; Van Handel, 1993 and Diss *et al.*, 1996). Lipids are considered to cover the energetic demands of the developing embryo, while proteins are mainly structural components, but may additionally serve as energetic resource (Beenakkers *et al*, 1985). For a long time, it was believed that Lepidoptera acquire all their nitrogenous reserves during their larval period, whereas adult feeding was believed to cover energy requirements only (Engelmann, 1970 and Wiggelsworth, 1972). Recently, Levin et al. (2017) found that both essential and nonessential amino acids were allocated to eggs and flight muscles in *Manduca sexta*. Additionally, the role of amino acids in nectar may differ between species (Wheeler and Buck, 1996). Such differences suggest that reproductive resource allocation is a rather complex issue and that any generalizations about the role of adult diet-derived amino acids for butterfly reproduction seem premature. Yeast is known to be an excellent source of protein to insect. The fermenting activity of yeasts results in the production of noticeable concentrations of e.g. ethanol (Leavey, 2004). Ethanol at low concentrations may serve as an energy source (apart from being an olfactory cue; e.g. Omura and Honda (2003), while high concentrations (45.0–7.5%) are toxic (Heberlein *et al.*, 2004).

Lipids are likely to be of key importance for insect reproduction, as they are major constituents of the oocyte dry mass and serve various functions including their role as the main energy source for the developing embryo (Ziegler and Van Antwerpen, 2006). As most insects are neither able to synthesize long-chain polyunsaturated fatty acids (hereafter PUFAs; but Beenakkers *et al*., 1985) nor the tetracyclic steroid nucleus required for the synthesis of sterols (Behmer and Nes, 2003) de novo, they depend on exogenous sources for successful development and reproduction (Al-Izzi and Hopkins, 1982; Beenakkers *et al.*, 1985; Turunen, 1990; Behmer and Grebenok, 1998; Svoboda, 1999 and Mondy and Corio-Costet, 2000).

In conclusion, when interpreting these findings, it should be borne in mind that both larval and adult diets seem to strongly interact with each other to fully meet all nutritional requirements of insects. And conclude that the non-carbohydrate components in adult's diet may play important roles in both reproductive success and survival of pink bollworm. Generally, the performance of the moths on the oil diet was consistently good. It enabling the moths to live moderately and produce more eggs, the oil diet are also relatively inexpensive; thus, they can be considered good diets for maintaining laboratory colonies of these species of moths.

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