

Impact of numerous larval diets on the biology of pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) under laboratory conditions

AKHTAR Shamim^{1,2*}, ARIF Muhammad Jalal¹, GOGI Muhammad Dildar¹ and HAQ Imran-ul³

Abstract

Background Pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) has become a potential pest of cotton by causing substantial yield losses around the world including Pakistan. Keeping in view the facts like limited research investigations, unavailability, and high cost of artificial diet's constituents and their premixes, the present research investigations on the dietary aspect of *P. gossypiella* were conducted. The larvae of *P. gossypiella* were reared on different diets that were prepared using indigenous elements. The standard/laboratory diet comprised of wheat germ meal 34.5 g, casein 30.0 g, agar–agar 20.0 g, sucrose 10.0 g, brewer's yeast 5.0 g, α-cellulose 1.0 g, potassium-sorbate1.5 g, niplagin 0.5 g, decavitamin 0.01 g, choline-chloride 0.06 g, maize-oil 3.30 g, honey 2.0 g, and water 730.0 mL. Alternatives to cotton bolls and wheat germ meal were okra seed sprouts, okra fruit, cottonseed meal, and okra seed meals, which were included in the study to introduce an efficient and economic mass-rearing system.

Results The larval development completed in 19.68 d \pm 0.05 d with a weight of 20.18 mg \pm 0.20 mg at the fourth instar fed on the cottonseed meal-based diet instead of wheat germ meal based diet. On the same diet, 84.00% \pm 4.00%, 17.24 mg \pm 0.03 mg, and 7.76 d \pm 0.06 d were recorded as pupae formation, pupal weight, and pupal duration, respectively. Adult emergence, 76.00% \pm 1.00% was recorded from pupae collected from larvae raised on cottonseed meal-based diet. These male and female moths lived for 40.25 d \pm 0.10 d, and 44.34 d \pm 0.11 d, respectively. Females deposited 21.28 \pm 0.04 eggs per day with the viability of 65.78% \pm 0.14%. The larval mortality at the fourth instar was 37.20% \pm 1.36% and malformed pupation of 12.00% \pm 1.41% was recorded. Replacement of wheat germ meal with that of local meals (cottonseed and okra seed) in the standard laboratory diet has saved 463.80 to 467.10 PKR with 1.62 to 1.63 cost economic returns, respectively.

Conclusion This research is of novel nature as it provides a concise and workable system for the economic and successful rearing of *P. gossypiella* under laboratory conditions.

Keywords Artificial diets, Cottonseed meal, Laboratory rearing, *Pectinophora gossypiella*, Wheat germ meal, Indigenous elements, Economic mass production, Pink bollworm

*Correspondence: Akhtar Shamim ashamim31@gmail.com Full list of author information is available at the end of the article



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Background

Pink bollworm *Pectinophora* gossypiella (Saunders) (Lepidoptera: Gelechiidae) is amongst the most destructive insect pests of cotton around the globe. Pakistan has also suffered huge losses in cotton for quantity and quality during the recent couple of decades because of P. gossypiella attack (Ali et al., 2016; Razzag et al., 2021; Abbas et al., 2022). P. gossypiella is known to cause 2.8% to 61.9% loss in seed cotton yield, 2.1% to 47.1% loss in oil content, and 10.7% to 59.2% boll opening loss (Patil et al., 2004). There was a transformational era in global cotton production with the introduction and commercial adoption of transgenic cotton cultivation during 1996. Globally, the total area under cotton cultivation was 35 million hectares out of which 22.3 million hectares were sown with transgenic Bacillus thuringiensis gene (Bt) cotton (James, 2010). Transgenically modified crops produce toxin proteins that offer a high degree of resistance against cotton bollworm complex in the laboratory and field conditions (Kranthi, 2002; Kranthi et al., 2004). However, the investigation on the resurgence of P. gossypiella proved the evolution of resistance against genetically modified cotton (Fabrick et al., 2015). Such scientific investigations demand a sustainable availability of the insect pests to explore various phenomena involved. The resistance monitoring research becomes more difficult to execute mainly because of complications in the mass-rearing facilities under laboratory conditions (Reyaz et al., 2018).

The successful mass production system mainly depends upon the availability of alternative artificial diets prepared from different compounds, following standardized protocols with the elimination of importation of costly diet premixes and essential apparatus. Several attempts have been made in the past using various elements as larval and adult diets for mass production of *P. gossypiella* in laboratory conditions (Vanderzant et al., 1956; Vanderzant et al., 1956a; Vanderzant et al., 1956b; Adkisson, 1961; Raulston, 1971; Patana, 1977; Miller et al., 1996; Muralimohan et al., 2009; Dharajothi et al., 2016). The use of natural hosts as diet (larvae and adults) for rearing of P. gossypiella under controlled conditions makes the rearing system non-economic and non-feasible because of the expensive inputs to maintain a regular and well-in-time supply of the food stuff. Moreover, field-collected natural hosts become a source of infection to the laboratoryreared population of insects. Hence, it results in the need for alternative insect food sources. Previously developed commercial diets are expensive and require a lot of time and effort making laboratory rearing of P. gossypiella more difficult. Indigenous elements thus are preferred to incorporate in the final formulation for developing diets in a cost-effective rearing facility. Keeping in view all the issues and to develop a reliable mass-rearing system, several elements (natural and artificial) were tested to study their effect on various biological aspects of P. gossypiella when used as food for the larval stage. The screening of such ingredients will not only help to find the most suitable food source among the indigenous constituents but objective-oriented utilization also.

Methods

Starter culture and population homogenization

Pink bollworm *P. gossypiella* infested fruiting bodies (flowers, squares, and bolls; Fig. 1) were collected from three different locations in Faisalabad. These locations were: 1) Young Wala, farmer's fields (31°26′05″ N, 73°03′45″ E), 2) Ayub Agricultural Research Institute, field area (31°24′11″ N, 73°02′54″ E), and 3) Jhipal chak no. 73 field area (31°21′37″ N, 72°52′16″ E) where different Bt-cotton varieties were sown. The infested samples were brought in the pink bollworm rearing laboratory, Department of Entomology, University of Agriculture, Faisalabad.

The larvae from all infested materials were isolated and reared on 10–20-days old freshly picked green cotton bolls (Naik et al., 2021). The bolls were washed with tap water, blotted dry, and pricked with a sterilized



Fig. 1 Pectinophora gossypiella infested cotton flowers (a), squares (b), and bolls (c)

surgical needle before providing field-collected larvae of *P. gossypiella* (Fabrick et al., 2015). Pricking of the green bolls was done to facilitate the entry of larvae inside the bolls. Pupae of the same age were collected in a glass petri dish lined with a layer of paper towel. The petri dish containing pupae was placed in adult chambers. Glass chimneys were set as emergence chambers for adults. The top and bottom of the adult chambers were lined with a layer of oviposition substrate (Sabry et al., 2013), and 10% (mass fraction) sucrose solution was provided as an adult diet. In each chamber, fifteen moth pairs (male to female ratio was 1:1) of *P. gossypiella* were released which were identified at larval and pupal stages during development (Dharajothi et al., 2010).

Preparation of standard laboratory larval diet and experiment layout

The standard laboratory diet (control treatment/ T_7) was prepared by performing the subsequent steps as follows:

- 1. Agar–agar 20 g (Table 1, serial No. 5) was dissolved in 500 mL distilled water (Table 1, serial No. 15) in a liter 1 000 mL glass beaker. The mixture is then boiled for 3–4 min in an oven with continuous stirring and heating at 30 s intervals to avoid flocculation.
- 2. The remaining amount of distilled water, 230 mL (Table 1, serial No. 15) was added in the blending jug (electric blending machine).
- 3. The ingredients (Table 1, serial No. 3 to 14 excluding agar–agar) were accurately weighed, and added to distilled water mentioned at step No. 2.
- 4. The hot boiling mixture of agar–agar prepared at step No. 1 was poured into the blender contents mentioned at step No. 3.
- 5. The final mixture was blended continuously for 2–3 min with intermittent pausing.
- 6. The final uniform mixture (standard laboratory diet) was poured into glass petri-dishes previously sterilized, and the diet was cooled at room temperature for 30 min.
- 7. Once the diet in petri dishes solidified, it was sliced into pieces of different sizes according to the requirement of the experiment (usually 0.50 cm^3) with the help of a knife.
- 8. The test diets for the treatments $(T_1 \text{ and } T_2)$ were prepared by following the same protocol as for the standard laboratory diet (T_7) (Fig. 2a and 2b), respectively. The difference is only that wheat germ meal in the standard laboratory diet (T_7) (Fig. 2c) was replaced with cottonseed meal and okra seed meal

(Table 1, Serial No. 1 and 2) for test diets in T_1 and T_2 , respectively.

9. The sliced diet was presented to the larvae of *P. gossypiella* in plastic cups (Fig. 3) (Wu et al., 2008).

Before larval diet preparation, a stock solution of decavitamin was prepared by weighing all the constituents (Table 2). The ingredients were added in a glass vial containing distilled water. The final mixture of decavitamin was made by vigorous shaking for 2-3 min. The mixture of decavitamin was stored at 20 °C in a refrigerator for further use.

The seed sprouts of cotton and okra to be used as test larval diets (treatment T₃ and T₄) were prepared by following predefined procedures (Vanderzant et al., 1956) (Fig. 2d). The seeds of cotton (FH-301) and okra (Sabz Pari) were obtained from Punjab Seed Corporation, Ayub Agricultural Research Institute, Faisalabad, for preparing their sprouts. Cotton bolls (FH-301), 10-20days old were picked from the field area. The bolls were washed and disinfected with formalin (mass fraction 0.1%) by dipping in for 2-3 min to avoid secondary infection. The traces of formalin were removed by subsequent washings 2-3 times and dried at room temperature (Fand et al., 2020). These bolls were dried with a paper towel and left for 5 min then offered to the larvae as diet (treatment, T_5) (Fig. 2e). Similarly, okra fruit was sliced in (1/2'', about 12.7 mm) pieces on a paper to remove mucilaginous material as the larvae may not stick in and become immobile in the corresponding

 Table 1
 Ingredients of artificial larval diets for the larvae of Pectinophora aosypiella

Sr. No	Ingredients	Brand/Variety	Quantities
1	Cottonseed meal	FH-301	34.50 g
2	Okra seed meal	Sabz Pari	34.50 g
3	Wheat germ meal	Solarbio	34.50 g
4	Casein	UNI-CHEM	30.00 g
5	Agar–agar	Solarbio	20.00 g
6	Sucrose	SCP	10.00 g
7	Brewer's yeast	SCP	5.00 g
8	a-cellulose	SCR	1.00 g
9	Potassium sorbate	Solarbio	1.50 g
10	Niplagin	Solarbio	0.50 g
11	Decavitamin	-	0.01 mL
12	Choline chloride	SCP	0.06 g
13	Maize oil	Rafhan	3.30 mL
14	Honey	Langnese	2.00 mL
15	Distilled water	-	730.00 mL



Fig. 2 Cottonseed meal based larval diet (**a**), okra seed meal based larval diet (**b**), wheat germ meal based larval diet (**c**), seed cotton sprouts (**d**), cotton bolls (**e**), and okra fruits (**f**) for *Pectinophora gossypiella*

treatment (T_6) (Fig. 2f). The detail of the different diets according to treatments was tabulated (Table 3).

First instar larvae were released in plastic cups according to the treatments, labeled and placed in a larval rearing chamber with controlled conditions maintained at (27 ± 0.5) °C (Dharajothi et al., 2016; Shrinivas et al., 2019), $60\% \pm 5\%$ relative humidity, and 12:12 (L: D) photoperiod (Reyaz et al., 2018).

The diets in treatments T₁, T₂, and T₇ were sliced into 0.50 \mbox{cm}^3 and 2–3 pieces were placed in each cup as larval diet. The diets in all treatments were refreshed after 48 h except T₅ (Freshly picked green cotton bolls) until the completion of the larval development (Fig. 4a). Those larvae that pupated on the same day were separated from those still in the larval phase and placed in a petri dish lined with a layer of tissue paper to avoid trauma (Fig. 4b). Adults emerging from pupae were placed in adult chambers (glass chimney cages) and, provided with 10% (mass fraction) honey solution as adult food (Hariprasad, 1999; Wu et al., 2006) (Fig. 4c). The adults were held at (27 ± 2) °C, 60%–70% relative humidity, and 14:10 (L: D) photoperiod (Wu et al., 2013; Malthankar et al., 2014; Zinzuvadiya et al., 2017; Fand et al., 2020). The egg receptacles were removed from adult chambers (Fig. 4d), incubated in transparent plastic boxes ($12.5 \text{ cm} \times 4.5 \text{ cm} \times 2.5 \text{ cm}$) with air-tight lids, and kept at (28 ± 1) °C (Muralimohan et al., 2009).

The experiment was executed following the completely randomized design (CRD) as one larva/cup to avoid cannibalism and seven treatments. For adults, fifteen moth pairs (male and female ratio was 1:1) were released in each adult chamber (glass chimney cage) that represented one replication.

The cost of all diets' ingredients for individual combinations in a single preparation was calculated and compared to determine cost economic returns while preferring one combination over others in the mass production of *P. gossypiella*.

Statistical analysis

Data on biological characteristics like larval longevity (duration before the larvae pupate), larval weight, pupal weight, pupae formation, pupal duration, adult emergence, and male and female life span were recorded for three years. The data on the fecundity, egg viability, larval mortality, pupation, and deformed pupation in the decedents were also recorded. The



Fig. 3 Plastic cups with the standard laboratory diet for *Pectinophora gossypiella* larvae

 Table 2
 Decavitamin (ingredients) of larval diet for Pectinophora gossypiella larvae

Sr. No	Ingredients	Brand/Variety	Quantities
1	Calcium pentothenate	Solarbio	12.00 mg·mL ^{−1}
2	Niacin	Sigma aldrich	6.00 mg·mL ⁻¹
3	Riboflavin	Sigma aldrich	3.00 mg·mL ⁻¹
4	Folic acid	Sigma aldrich	3.00 mg·mL ⁻¹
5	Thiamine	Sigma aldrich	1.50 mg·mL ⁻¹
6	Pyridoxine	Solarbio	0.93 mg·mL ⁻¹
7	Biotin	Solarbio	1.50 mg·mL ⁻¹
8	Vitamin B_{12}	Sigma aldrich	0.012 mg·mL ^{−1}

collected data were statistically analyzed using the statistical software Statistica to check the significance of the results at the 5% probability level and means of each parameter were compared by Tukey's HSD. The calculation of the cost economic return/benefit ratio was performed by simple mathematical formulae.

Results

Larval longevity/duration before the larvae pupate

Among all the tested larval diets (artificial and natural), the larvae took longest duration to complete development (19.68 d \pm 0.05 d) when reared on diet having cottonseed meal as a replacement for wheat germ meal in the standard laboratory diet. This period found to be statistically at par (19.48 d \pm 0.20 d) with those when the larvae were provided with okra seed sprouts as food. Among the artificial formulations, the larvae completed development (1st instar to 4th instar) in 16.48 d \pm 0.09 d when wheat germ meal of the standard laboratory diet was replaced with okra seed meal. However, among the natural food sources (cottonseed sprouts, okra seed sprouts, cotton bolls and okra fruit) the larvae completed development (1st to 4th instar) in the shortest duration (10.48 d \pm 0.16 d) when fed on cotton bolls or okra fruit (Table 4).

Larval and pupal weight

The larvae and pupae gained maximum weight $(26.51 \text{ mg} \pm 1.07 \text{ mg}, 20.13 \text{ mg} \pm 0.05 \text{ mg})$ when fed on cotton bolls while minimum $(16.52 \text{ mg} \pm 0.06 \text{ mg}, 14.53 \text{ mg} \pm 0.08 \text{ mg})$ on cotton seed sprouts, respectively (Table 4). The larvae and pupae gained (20.18 mg $\pm 0.20 \text{ mg}, 17.24 \text{ mg} \pm 0.03 \text{ mg})$ weight when supplied with an artificial diet containing cottonseed meal instead of wheat germ meal, respectively (Table 4).

Pupae formation and pupal duration

Pupae formation was highest (96.00% \pm 4.00%) when cotton bolls were used as a larval diet which was statistically similar (84.00% \pm 4.00%) to that recorded on the cottonseed meal based diet (Table 4). The pupal duration recorded in all test diets (artificial and natural) was categorized into two groups with maximum and minimum duration (8.64 d \pm 0.03 d, 7.68d \pm 0.21 d) from the pupae whose larval diet was okra fruit and cotton seed sprouts, respectively (Table 4).

Adult emergence

Among all the test larval diets (artificial and natural) the highest numbers of adults (90.00% \pm 6.12%) emerged when okra seed sprouts were provided as larval food while the lowest (57.67% \pm 8.68%) on cottonseed sprouts (Table 4). The adult emergence (76.00% \pm 1.00%) recorded when the cottonseed meal based larval diet was provided was statistically at par with that recorded on cotton bolls (Table 4).

Life span of male and female

The shortest life span of male and female moths $(36.80 \text{ d} \pm 0.05 \text{ d}, 41.25 \text{ d} \pm 0.07 \text{ d})$ was recorded when cotton bolls were provided as food during the larval stage, respectively (Table 4). Among the artificial test diets, male and female *P. gossypiella* completed their development in 40.25 d ± 0.10 d, 46.77 d ± 0.07 d with

Treatments	Diet ingredients	Quantities
T ₁	Cottonseed meal, casein, agar-agar, sucrose, brewer's yeast, α-cellulose, potassium-sorbate, niplagin, decavitamin, choline-chloride, maize-oil, honey and distilled water	As specified in Table 1
T ₂	Okra meal, casein, agar–agar, sucrose, brewer's yeast, α-cellulose, potassium-sorbate, niplagin, decavitamin, choline-chloride, maize-oil, honey and distilled water	As specified in Table 1
T ₃	Seed sprouts of cotton (FH-301 variety)	3–4 seed sprouts per larvae/cup
T ₄	Seed sprouts of okra (Sabz Pari variety)	3–4 seed sprouts per larvae/cup
T₅	Freshly picked green cotton bolls (FH-301 variety)	One boll per larvae/cup
T ₆	Okra fruit (Sabz Pari variety)	3–4 okra fruit slices per larvae/cup
T ₇	Wheat meal, casein, agar–agar, sucrose, brewer's yeast, α-cellulose, potassium-sorbate, niplagin, decavitamin, choline-chloride, maize-oil, honey and distilled water	As specified in Table 1

Table 3 Larval diets for the larvae of Pectinophora gossypiella

cottonseed (male) and okra seed meal (female) based larval diets, respectively (Table 4).

Fecundity and egg viability

The female moths deposited the highest number of eggs per female $(22.26 \pm 0.01 \text{ eggs} \cdot \text{d}^{-1})$ when okra fruit was provided as a larval diet, but the viability of the eggs was the lowest $(49.42\% \pm 0.02\%)$ (Table 4). Contrasting to it, the fecundity and egg viability per female were 21.28 ± 0.04 and 18.32 ± 0.09 eggs·d⁻¹, and $65.78\% \pm 0.14\%$ and $76.41\% \pm 0.37\%$ when cottonseed and okra seed meal based larval diets were provided, respectively (Table 4).

Larval mortality and pupation

The eggs deposited from the female moths of *P. gos-sypiella* were collected (all the test larval diets) and incubated. The neonates were provided with the same larval diets as that of their parents. These larvae were observed till pupation. It was found that lower mortality $(37.20\% \pm 1.36\%)$ with higher larval

pupation ($62.80\% \pm 1.36\%$) was noted when cottonseed meals (among artificial diets) based diet was supplied (Table 4).

Deformed pupation

The maximum and minimum of malformed or deformed pupation was $12.00\% \pm 0.63\%$ and $6.80\% \pm 0.49\%$ when cotton seed sprouts and okra seed meal based larval diets were provided, respectively (Table 4).

Cost-benefit analysis

The analysis of the results for cost using a previously developed diet/standard for laboratory rearing of pink bollworms showed financial benefits over using indigenous sources. The advantages of the use of locally available diets' ingredients or diets included easy and quick availability at comparatively lower prices with little effort than to import diet constituents or premixes at high prices making the rearing system more efficient and economical (Table 5).



Fig. 4 Pectinophora gossypiella larvae (a), pupae (b), adults (c), and eggs (d)

		Meal based diet (Cottonseed) T ₁	Meal based diet (Okra seed) T ₂	Cotton seed sprouts T ₃	Okra seed sprouts T ₄	Green Cotton bolls T ₅	Okra fruit (slices) T ₆	Control/Meal based diet (Wheat germ) T ₇
_	Larval longevity /d	19.68 ± 0.05^{a}	16.48±0.09 ^b	16.15±0.79 ^b	19.48 ± 0.20^{a}	10.48±0.16 ^d	10.48±0.16 ^d	13.10±0.28 ^c
2	Larval weight /mg	20.18±0.20 ^b	18.11 ± 0.07^{cd}	16.52 ± 0.06^{d}	17.18 ± 0.03^{d}	26.51 ± 1.07^{a}	20.08 ± 0.03^{b}	19.56±0.14 ^{bc}
e	Pupal weight /mg	$17.24 \pm 0.03^{\circ}$	15.52 ± 0.09^{d}	14.53 ± 0.08^{f}	15.23 ± 0.02^{e}	20.13 ± 0.05^{a}	18.39 ± 0.05^{b}	17.14 ± 0.06^{c}
4	Pupae formation /%	84.00 ± 4.00^{ab}	64.00±4.00 ^b	80.00 ± 6.32^{ab}	68.00 ± 8.00^{b}	96.00 ± 4.00^{a}	72.00±4.90 ^b	76.00±4.00 ^{ab}
5	Pupal duration /d	7.76 ± 0.06^{b}	8.53 ± 0.03^{a}	7.68±0.21 ^b	8.62 ± 0.05^{a}	7.73±0.03 ^b	8.64 ± 0.03^{a}	8.02 ± 0.12^{b}
9	Adult emergence /%	76.00 ± 1.00^{ab}	75.00 ± 6.45^{ab}	57.67 ± 8.68^{b}	90.00 ± 6.12^{a}	88.00 ± 4.90^{a}	78.33 ± 5.65^{ab}	88.33 ± 7.26^{a}
7	Male life span /d	40.25 ± 0.10^{d}	38.43 ± 0.02^{f}	42.38 ± 0.04^{a}	40.90 ± 0.02^{c}	36.80 ± 0.05^{9}	41.44±0.01 ^b	38.93 ± 0.03^{e}
8	Female life span /d	44.34 ± 0.11^{f}	46.77±0.07 ^{de}	$48.56 \pm 0.19^{\circ}$	50.24 ± 0.08^{b}	41.25 ± 0.07^{9}	48.23 ± 0.06^{cd}	54.46 ± 0.03^{a}
6	Fecundity / (eggs·female ^{-1.d–1}	21.28±0.04 ^b	18.32±0.09 ^d	14.54 ± 0.04^{f}	12.21±0.02 ⁹	16.55±0.19 ^e	22.26 ± 0.01^{a}	19.22±0.07 ^c
10	Egg viability /%	65.78±0.14 ^e	76.41±0.37 ^c	96.29±0.27 ^b	98.31 ± 0.14^{a}	67.68 ± 0.60^{d}	49.42 ± 0.02^{9}	57.22 ± 0.20^{f}
11	Larval mortality /%	37.20±1.36 ^{de}	40.80±0.49 ^{bc}	43.60 ± 0.40^{ab}	34.40 ± 0.40^{e}	39.60 ± 0.75^{cd}	45.20 ± 0.49^{a}	12.80 ± 0.49^{f}
12	Larval pupation /%	$62.80 \pm 1.36^{\circ}$	59.20 ± 0.49^{e}	56.40 ± 0.40^{f}	65.60 ± 0.40^{b}	60.40 ± 0.75^{d}	54.80 ± 0.49^{9}	87.20 ± 0.49^{a}
13	Deformed pupa- tion /%	12.00±1.41 ^a	6.80 ± 0.49^{d}	12.00 ± 0.63^{a}	10.80 ± 0.80^{b}	7.60 ± 0.40^{c}	5.20±0.49 [€]	2.40±0.40 ^d

Table 4 Biological parameter (means ± standard error) recorded on various types of larval diets of Pectinophora gosspiella under laboratory conditions

Biological Parameter Treatments (Diets) Sr. No

Means in the same rows following the same letters are statistically similar (P>0.05)

Treatments	Description	Cost of Diet / preparation / PKR	Cost saving / PKR	Cost economic return
T ₁	Cottonseed meal replacing wheat germ meal in the standard diet	741.70	463.80	1.62
T ₂	Okra seed meal replacing wheat germ meal in the standard diet	738.40	467.10	1.63
T ₃	Seed sprouts of cotton (FH-301 variety)	170.25	1 035.25	7.08
T ₄	Seed sprouts of Okra (Sabz Pari variety)	142.50	1 063.00	8.46
T₅	Freshly picked green cotton bolls (FH-301 variety)	86.00	1 119.50	14.02
T ₆	Okra fruit (Sabz Pari varieity)	45.00	1 160.50	26.79
T ₇	Wheat germ meal based diet (standard diet)/control	1 205.50	-	-

Table 5 Cost economic return/benefit of using various oviposition substrates

Calculations: The value of US \$ 1.00 = 138.44 Pakistan Rupees (during the import period of the chemicals)

The value of (\pounds) 1.00 = 186.14 Pakistan Rupees (during the import period of the chemicals)

Cost saving compared to control = cost in control - cost in treatment

Cost economic return = cost in control/ cost in treatment

Discussion

Larval longevity/duration before the larvae pupate

The result of the present investigation for larval longevity 19.68 d \pm 0.05 d contradicts those 25.01 d \pm 0.994 d and 26.10 d±3.76 d by Dharajothi et al. (2016) and Muralimohan et al. (2009), respectively. The potential reasons behind this deviation might be the varietal difference of cottonseed meal-based diets (either Bt or non-Bt) and the rearing conditions followed by holding containers. However, larval longevity of 13.10d ±0.28 d recorded (wheat germ meal-based larval diet) in our study resembles those reported by Adkisson et al. (1960) who recorded the larval longevity of 1.30–26.00 d using the same diet. Comparatively, the larvae developed faster than other tested diets and pupated in 10.48 ± 0.16 d when reared on green bolls or excised pieces of okra fruit. The results for minimum larval longevity (10.48 d \pm 0.16 d) of the present study are approximately similar (8.03 d \pm 0.43 d to 11.36 d \pm 0.30 d) with those reared on the same diet (cotton bolls) reported by Rajput et al. (2019).

Larval and pupal weight

The larvae gained maximum and minimum weight of 26.51 mg ± 1.07 mg and 16.52 mg ± 0.06 mg when reared on freshly picked bolls of non-Bt cotton (FH-301) variety and its seed sprouts, respectively. These findings contradict those reported by Rajput et al. (2019) who reared larvae on non-Bt cotton and Bt cotton varieties with comparatively less weight 20.24 mg ± 1.74 mg and 13.84 mg ± 1.30 mg, respectively. This difference in larval weight may either be the difference in cotton variety or the influence of rearing containers. Whereas, larvae gained 20.18 mg ± 0.20 mg weight when provided with a cotton-seed meal based diet in this study. This result is similar to those reported by Dharajothi et al. (2016) who recorded 21.4 mg ± 3.63 mg using the same primary constituent.

The results for the pupal weight (20.13 mg \pm 0.05 mg) of the present study are different from the pupal weight 23.46 mg \pm 0.55 mg when reared on cotton bolls of different non-Bt varieties Rajput et al. (2019). However, the lowest pupal weight of 14.53 mg \pm 0.08 mg was recorded when the larvae fed on cotton seed sprouts. The variation in pupal weight (15.52 mg \pm 0.09 mg–17.24 mg \pm 0.03 mg) of the cottonseed meal based diet and okra seed meal based diet in the present study is approximately similar to those conducted by Muralimohan et al. (2009) 14.75 mg \pm 2.81 mg–21.78 mg \pm 4.09 mg, Adkisson et al. (1960) 20.50–23.00 mg wheat germ meal based diet and Vanderzant et al. (1956a) 12.00–15.20 mg using the casein-based diet.

Pupation and pupal duration

The highest pupae formation of $96.00\% \pm 4.00\%$ was recorded when larvae reared on freshly picked bolls (FH-301) variety which is similar to those (95.33%) obtained by Reyaz et al. (2018) reared on cotton bolls (MCU-13 variety). Among the seed meal based diets, minimum (64.00% ± 4.00%) pupae formation was recorded for okra seed meal based diet. The results are in line with the investigations by Adkisson et al. (1960) when they obtained a success rate of up to 81.5% for adult recovery on a wheat germ meal based diet.

Adult Emergence

An adult emergence $(88.33\% \pm 7.26\%$ to $75.00\% \pm 6.45\%)$ was documented when seed-meal based diets were provided to the larvae of *P. gossypiella* which fall within the range of results reported by Dharajothi et al. (2016), Muralimohan et al. (2009), and Adkisson et al. (1960) who recorded 95.56\%, 62.06\% to 91.66\%, and 75.00% to 85.00% adult emergence. The adult emergence results obtained from larvae reared on natural diets (57.67\% \pm 8.68\% to

 $90.00\% \pm 6.12\%$) resemble those reported by Fand et al. (2020), $92.32\% \pm 2.34\%$ emergence.

Life span of male and female

The results of the present study for *P. gossypiella* male life cycle (40.25 d±0.10 d) do not conform to those reported by Zinzuvadiya et al. (2017) who recorded 38.40 d±4.48 d using the cottonseed meal based diet. The possible reason for the difference could be the variation in diet ingredients and the rearing equipment used in the experiments. As far as the results of the female life span (44.34 d±0.11 d) in the present investigation differ from those reported by Zinzuvadiya et al. (2017) who recorded 56.30 d±9.84 d while providing the cottonseed meal based diet. The possible reason for the contradiction could be the variation in diet ingredients as well as the rearing equipment.

Fecundity and egg viability

The average number of eggs deposited of *P. gos-sypiella* per famale was in the range of 22.26 ± 0.01 and 12.21 ± 0.02 eggs·d⁻¹ when different foods were provided during larval developmental stages. The results recorded in this research were different from those reported by Rajput et al. (2019) who recorded 8.04 ± 0.19 eggs· when studying the females on non-Bt cotton (during their larval development stage). Even the lowest female fecundity rate (12.21 ± 0.02 eggs·d⁻¹) recorded in this research is 1.52 times higher than those under discussion. Nevertheless, the egg viability ($49.42\% \pm 0.02\%$ to $98.31\% \pm 0.14\%$) upper range found was 1.22 times higher than previously reported by Raina et al. (1978) who recorded lower egg viability (65.20% to 80.70%).

Larval mortality and pupation

Mortality in the laboratory-reared strain larvae was recorded at $39.60\% \pm 0.75\%$ when fed on freshly picked green bolls (non-Bt cotton bolls). These results are different from that reported 24.95% by Rajput et al. (2019) using the same diet. The possibility of higher mortality (contradiction) could be differences in food because they used cotton bolls of Bt cotton rather than non-Bt cotton bolls. A wide range of pupae formation was recorded from the tested diets including seed meals based (artificial diet/meals-based diets) and natural hosts. The pupation recorded in these treatments ranged from $54.80\% \pm 0.49\%$ to $87.20\% \pm 0.49\%$. These results are different from those reported by Vanderzant et al. (1956a) who found pupation of 23.00% to 87.00% with purified casein media-based diets (addition or deletion of other ingredients).

Deformed pupation

Malformed pupation was found ranging from $2.40\% \pm 0.40\%$ to $12.00\% \pm 1.41\%$ when reared on mealbased diets (wheat germ meal and cottonseed meal). This is similar to those reported by Muralimohan et al. (2009) who also reported malformed pupation from 1.31% to 13.40%.

Conclusions

These findings provide practical interventions for economic and successful laboratory rearing of *P. gossypiella* up to many generations keeping the vigor of the culture.

Abbreviation

PKR Pakistani Rupees

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Authors' contributions

Akhtar S made significant contributions in the execution of the experiments, recorded the data, statistically analyzed, interpreted the results, and drafted the manuscript. Arif MJ and Gogi MD conceptualized, planned, designed, supervised the entire research work and checked the final draft. Whereas, Haq I supervised the phyto-pathological aspect throughout the performance of the experiment and reviewed the final draft.

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Availability of data and materials

All the data relevant to the present study are included in the article. Any further details related to the experiments conducted can be made available by requesting the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Manuscript has not been published, or submitted for publication elsewhere.

Competing interests

The authors declare that they have no conflict of interest related the content of this article.

Author details

¹Department of Entomology, University of Agriculture Faisalabad, Punjab, Pakistan. ²Ayub Agricultural Research Institute (Entomological Research Institute), Faisalabad, Punjab, Pakistan. ³Department of Plant Pathology, University of Agriculture, Faisalabad, Punjab, Pakistan.

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