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COMPARATIVE STUDY OF PINK BOLLWORM BIOLOGY ON ARTIFICIAL DIET AND OKRA

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ABSTRACT

The study investigated the comparative biology of pink bollworm (PBW) reared on artificial diet and okra under controlled laboratory conditions with the temperature (27 ± 2 °C), relative humidity (65 ± 5%), and a 14:10 hours light-dark photoperiod during 2021-22 at ICAR-Central Institute for Cotton Research (CICR), Nagpur. The results indicated no significant difference in egg incubation, larval, prepupal, pupal, and oviposition periods between PBW reared on artificial diet and okra. However, adult longevity, total life cycle duration, and oviposition-related parameters exhibited significant differences. The study also highlighted differences in prepupal and pupal periods, with significant variations in adult longevity. Furthermore, the research demonstrated variations in fecundity and the highest number eggs laid when reared on artificial diet (126.19 ± 13.47) as compared to okra (91.95 ± 6.57). A higher percentage of egg hatching (88.70%) was observed in eggs laid by adults that were fed on an artificial diet, in contrast to pink bollworms fed on okra (84.90 %). Similarly, the percentage of adult emergence and survival rate was greatest (89.64% and 87.48%, respectively) in pink bollworms nourished with an artificial diet compared to those fed on okra (81.34% and 74.16 %, respectively). The total life cycle duration was found to be longer on artificial diet (40.92±4.89) compared to okra (38.60±3.60), emphasizing the influence of diet on PBW development. Overall, this investigation provides valuable insights into the biology and development of pink bollworm in relation to different food sources.

Key words : Pink bollworm, Biology, Artificial diet, Okra, Survival rate.

Introduction

The global cotton area and production amount to 32.6 million hectares and 116.10 million bales, respectively (Cotton Outlook, 2020). India, standing out among the world's cotton-producing countries, holds the foremost position in terms of area with 129.57 lakh hectares and production of 371 lakh bales, each weighing 170 kg, boasting a productivity of 487 kg lint per hectare (CCI, 2021). The intricate insect pest spectrum of cotton encompasses over 1326 species reported worldwide (Hargreaves, 1948). However, in India, damage to the cotton crop is attributed to 130 different species of insects and mites. Notably, the bollworms, such as the American bollworm (*Helicoverpa armigera*), spiny bollworm (*Erias insulana*), spotted bollworm (*E. vitella*) and pink bollworm (*Pectinophora gossypiella*), pose a significant threat to cotton production (Parmar and Patel, 2016). Within the bollworm complex, the pink bollworm stands out as a major destructive insect pest of Bt cotton, causing substantial losses across India (Dhurua and Gujar, 2011; Naik *et al*., 2018). Beyond cotton, it feeds on a diverse range of plants worldwide, including okra, hibiscus, deccan

hemp, roselle, Indian mallow, country mallow, hollyhocks, rose mallows, kenaf, jamaica sorrel, milo, lucerne and other malvaceous plant species (Noble, 1969; Khidr *et al*., 1990). The shift from monophagy to oligophagy, along with its ability to survive on alternative hosts during the off-season, exacerbates the impact of this pest on Bt cotton.

To investigate this phenomenon, an experiment was conducted to ascertain whether the pink bollworm completes its life cycle on the malvaceous host, okra. Additionally, a comparative study of the biology of the pink bollworm was undertaken by rearing it on an artificial diet and okra. These investigations provide insights into the off-seasonal survival, propagation, and spread of the pink bollworm in major cotton-growing areas. Comparative biology studies contribute to understanding how the pink bollworm adapts to different host plants, shedding light on its life history and behaviour. This knowledge serves as a valuable resource for farmers and agricultural researchers, enabling them to make informed decisions regarding crop management practices and enhancing the sustainability of crop production.

Materials and Methods

The current study on the comparative biology of the pink bollworm, *P. gossypiella* (Saunders) (Gelechiidae: Lepidoptera) was conducted in the laboratory at ICAR-Central Institute for Cotton Research (CICR), Nagpur, Maharashtra. The investigation took place under controlled conditions, specifically at a temperature of 27 ± 2°C, relative humidity of 65 ± 5%, and a photoperiod of 14:10 hours (light: dark) during the 2021-22 season. Pink bollworm larvae were harvested from the field by gathering damaged bolls and these larvae were reared on their natural food until reaching the pupation stage. Subsequently, the pupae were sexed based on pupal characteristics, specifically the position of mid-ventral setae on the 9th and 10th abdominal segments in males and females, respectively. The distance between the genital pore and anal pore served as a reliable character for sex differentiation, with the female's distance being more than double that of the male (Dharjothi *et al*., 2010). Sexed pupae were then placed in emergence cages (45×45×60cm) to facilitate adult eclosion. For mating purposes, five pairs of freshly emerged adults were introduced into oviposition cages.

A small conical flask, filled with water, housed a delicate cotton twig adorned with tender leaves, squares, and small bolls within an oviposition cage. To facilitate the feeding of adult moths, a cotton swab soaked in a 10 per cent honey solution was suspended by a thread in the cage. Fresh food was supplied daily by replacing the swab. Following egg hatching, 100 neonate larvae were carefully transferred to separate plastic jars (16" diameter × 18" height) using a moist camel brush. Subsequently, the larvae were nurtured on various host materials, including artificial diet and tender okra fruits. Cotton seed based artificial diet was used in this experiment, which was prepared by the standardized procedure developed at ICAR- CICR, Nagpur by Jothi *et al*. (2016) for continuous rearing of pink bollworm. These jars were placed in a BOD incubator with a controlled temperature (27 ± 2°C) and relative humidity (65 ± 5%). The study involved recording observations on fecundity, percentage of egg hatching, larval period, pupal period, and adult longevity, along with adult emergence (%) and survival rate (%). Additionally, weight of different life stages was meticulously documented. All the data was analysed by paired t-test using the WASP-web agri statistical package (Jangam and Thali, 2004). Detailed observations and results for each biological stage are outlined below.

Results and Discussion

Biology of pink bollworm on different foods

Comparative biology of pink bollworm was studied in BOD incubator having controlled conditions *viz*., 27 ± 2°C temperature, 65 ± 5% RH and photoperiod of 14:10 hours (light: dark) in the laboratory during 2021-22 and the results found that there was no significant difference in egg incubation, larval, prepupal, pupal and oviposition period when pink bollworm reared on artificial diet and okra. However, there was significant difference in adult longevity, total life cycle of both male and female reared on artificial diet and okra (P=0.05) (Table 1).

An average egg incubation period of pink bollworm was 3.08 ± 0.78 days with a range of 2.0-4.5 days, when reared on artificial diet. However, incubation period of eggs obtained by the PBW adults reared on okra was 2.86 ± 0.63 days with a range of 2.0-3.5 days and there was no significant difference in egg incubation period of PBW reared on artificial diet and okra. Similar results found by Cacayorin (1993) and Mushtaq *et al*. (2021), with an average incubation period of 3.68 ± 0.09 and 3.83±0.57 days on cotton. Syed *et al*. (2011) where they found that incubation period of eggs of *E. vittella* was less on okra (2.30 ± 0.50 days) than cotton (3.00 ± 0.0 days). Whereas, Whereas, Zinzuvadiya *et al*. (2017) reported an average egg period of 4.90 ± 0.99 days on artificial diet, which was in contrary with the present findings. The difference in incubation period of pink bollworm might be due to the effect of abiotic factors such as temperature, photoperiod and relative humidity

Table 1 : Biology of pink bollworm on artificial diet and okra.

Life stages		Artificial diet (days)			Okra (days)			t - Test
		Mean ± SD	Range	Variance	Mean ± SD	Range	Variance	
Egg Incubation		3.08±0.78	2.0-4.5	0.704	2.86±0.63	2.0-3.5	0.472	NS
I instar		3.22±0.82	2.0-4.5	0.879	2.88±0.58	2.0-4.0	0.378	NS
II instar		4.25±0.53	3.0-5.0	0.313	4.19±0.47	3.0-4.5	0.250	NS
III instar		4.82±0.61	4.0-6.0	0.418	4.60±0.38	4.0-5.0	0.177	NS
IV instar	**Male**	5.51±0.76	4.5-7.0	0.658	5.32±0.51	4.5-6.0	0.313	NS
	Female	6.35±0.49	5.5-7.5	0.238	6.23±0.35	5.5-6.5	0.146	NS
Pre pupa	**Male**	1.56±0.28	1.0-2.0	0.084	1.45±0.27	1.0-2.0	0.073	NS
	Female	1.91±0.37	1.5-2.5	0.156	1.78±0.36	1.5-2.5	0.126	NS
Total larval period	**Male**	19.35±3.01	14.5-24.5	9.656	18.43±2.21	14.5-21.5	5.224	NS
	Female	20.55±2.83	16.0-25.5	8.400	19.67±2.14	16.0-22.5	4.661	NS
Pupal period	**Male**	8.07±0.40	7.5-8.5	0.188	7.89±0.43	7.5-8.5	0.194	NS
	Female	8.53±0.40	8.0-9.5	0.168	8.36±0.35	7.5-9.0	0.130	NS
Adult longevity	**Male**	9.07±0.73	7.5-10.0	0.683	8.02±0.40	7.5-8.5	0.175	2.776
	Female	10.12±0.85	8.5-11.0	0.898	9.10±0.41	8.0-9.5	0.200	2.776
Pre oviposition period**		3.07±0.29	2.5-3.5	0.044	2.79±0.25	2.5-3.0	0.037	2.776
Oviposition period**		2.60±0.41	2.0-3.5	0.074	2.38±0.31	1.5-3.0	0.009	NS
Post oviposition period**		4.45±0.76	2.5-5.0	0.559	3.64±0.42	2.5-4.0	0.081	2.776
Fecundity (#) **		126.19±13.47	108.0-149.0	113.252	91.95±6.57	54.0-78.0	24.686	2.776
Total life cycle	**Male**	39.56±4.92	31.5-47.5	26.419	37.21±3.67	31.5-42.0	14.213	2.776
	Female	42.28±4.86	34.5-50.5	25.584	39.99±3.53	34.5-44.5	13.085	2.776
Average life cycle		40.92±4.89	39.5-42.5	25.967	38.60±3.60	33.0-43.25	13.618	2.776

NS = Non significant.

at which the experiment was conducted.

There are four larval instars of pink bollworm and fourth instar male larvae can be differentiated easily based on the pair of black colour oval gonads visible on dorsal side of the abdomen. The pink bollworm first instar larvae took an average of 3.22 ± 0.82 and 2.88 ± 0.58 days on artificial diet and okra. Similarly, II instar took an average of 4.25 ± 0.53 and 4.19±0.47, III instar took 4.82 ± 0.61 and 4.60 ± 0.38 days, IV instar male took 5.51 ± 0.76 and 5.32±0.51 days, IV instar female took 6.35 ± 0.49 and 6.23 ± 0.35 days on artificial diet and okra, respectively. Prepupal period varied with a range of 1.0-2.0 days in male and 1.5-2.5 days in female reared on both artificial diet and okra. However, total male larval period was 19.35 ± 3.01 and 18.43 ± 2.21 days, and total female larval period was 20.55 ± 2.83 and 19.67 ± 2.14 days on artificial diet and okra, respectively. There was no significant difference between the larval period of individual instars and total larval period on both the foods (Table 1). These results are in line with Fand *et al*. (2019) and Shah *et al*. (2013), where they found the total larval period of pink bollworm was 21.50 ± 1.04 and 21.50 ± 1.04 days on cotton. Similarly, Zinzuvadiya *et al*. (2017) found that the total larval period of pink bollworm ranged from 15-21 and 15-23 days in male and female, respectively. Whereas, the present findings are in contrary with Ramya Sri and Uma Maheshwari (2021) and Mushtaq *et al*. (2021) where they reported the average larval period of pink bollworm was 24.90 ± 1.42 and 26.38 ± 2.64 days this difference in the larval period may be due to the due to the effect of abiotic factors such as temperature, photoperiod and relative humidity at which the experiment was conducted.

The PBW male pupal period varied between 7.5-8.5 days with an average of 8.07 ± 0.40 and 7.89 ± 0.43 days on artificial diet and okra, respectively. Whereas, female pupal period varied between 8.0-9.5 days on artificial diet and 7.5-9.0 days on okra. The mean pupal

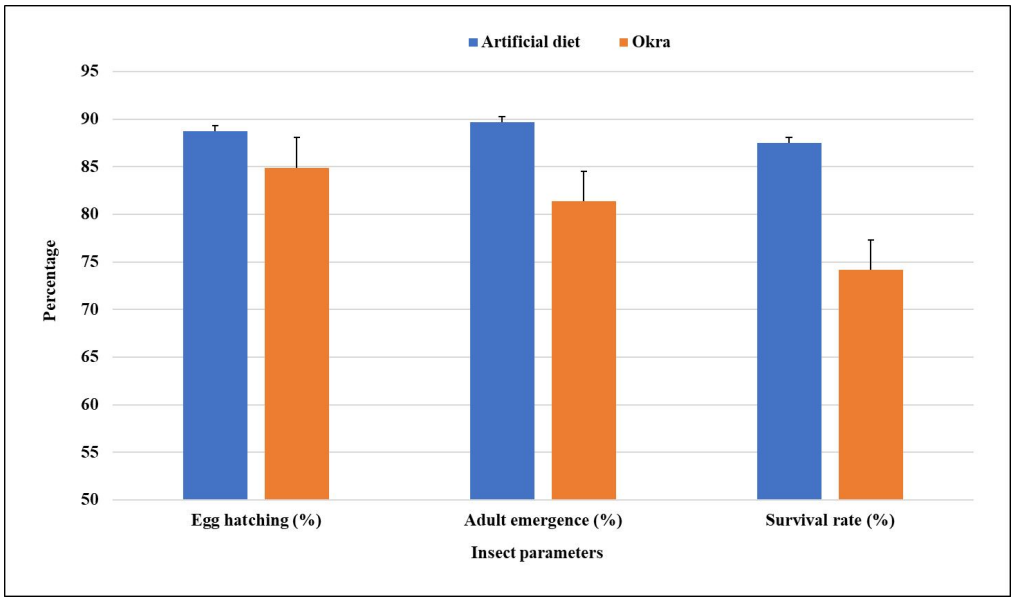

Fig. 1 : Pink bollworm egg hatching, adult emergence and survival rate when reared on artificial diet and okra.

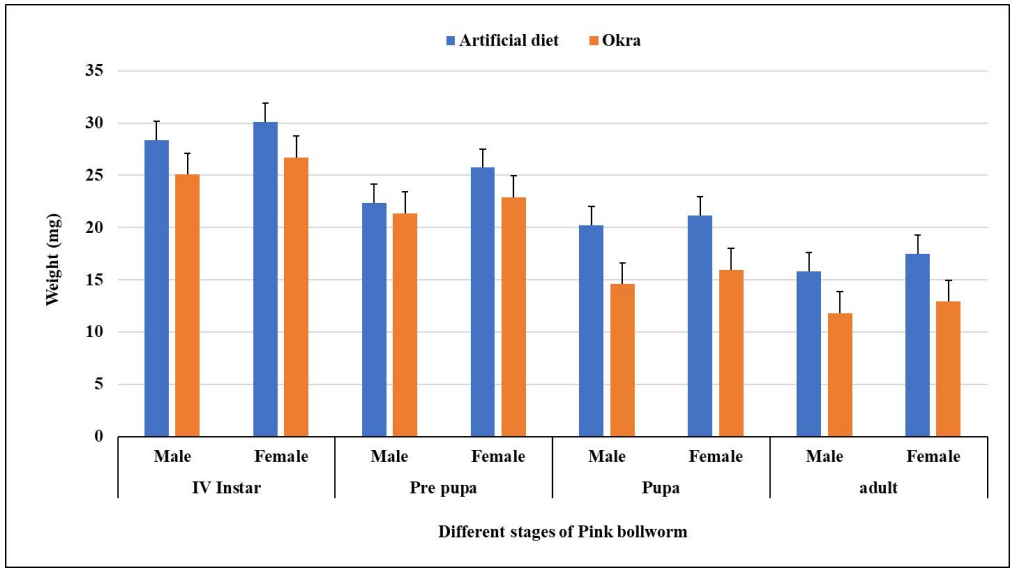

Fig. 2 : Effect of different food sources on weight of pink bollworm life stages (except male prepupa, weight of different stages on PBW reared on artificial diet and okra were statistically significant at p=0.05 with the t-test value 2.262).

duration of PBW on malvaceous plant okra was less than the artificial diet, these results are in indistinguishable with the results obtained by Shrinivas *et al*. (2019), who recorded that pupal period of PBW was shorter on okra (8.14 ± 0.29 days) than cotton (8.43 ± 0.18 days). However, Ramya and Uma (2021) notice an average pupal duration of 7.50 ± 1.00 and 7.25 ± 0.25 days with a range of 6.50-8.50 and 7.00-7.50 days on okra and abutilon.

The both male and female pink bollworm adult showed a significant difference (P=0.05) with respect to longevity. The average longevity of adult male was 9.07 ± 0.73 and 8.02 ± 0.40 days, and adult female was 10.12 ± 0.85 and 9.10 ± 0.41 days on artificial diet and okra, respectively. Similarly, Shrinivas *et al*. (2019) recorded the mean of 9.04 ± 0.18 and 9.85 ± 0.31 days with a range of 8.50-9.50 and 8.00-11.50 days in male and female moth, respectively in and Mushtaq *et al*. (2021) observed an average of 8.90 ± 0.81 and 9.97 ± 1.48 days in male and female moth on *Bt*-cotton.

The pre ovipositional period was ranged from 2.5 to 3.5 days with an average of 3.07 ± 0.29 days on artificial diet and 2.5-3.0 days with an average of 2.79 ± 0.25 days on okra. However, post ovipositional period was 4.45 ± 0.76 days on artificial diet and 3.64 ± 0.42 days on okra (Table 1).

The fecundity of pink bollworm ranged from 108.0-149.0 with an average of 126.19 ± 13.47 when reared on

artificial diet. However, on okra ranged from 54.0-78.0 with an average of 91.95 ± 6.57. The highest number eggs laid when reared on artificial diet as compared to okra and which were statistically significant at P=0.05 level of significance (Table 1). The present findings are analogous with Mushtaq *et al*. (2021) as they recorded an average 111.5 ± 13.96 eggs per female of pink bollworm on *Bt*-cotton. In contrast to the present findings, Shrinivas *et al*. (2019) reported an average fecundity of 118.65 ± 16.81 with a range of 100-185 eggs per female in okra. Likewise, Ramya and Uma (2021) observed an average fecundity of 124.50 ± 10.90 and 132.50 ± 10.50 eggs per female with a range of 113-135 and 122-143 eggs per female on okra and abutilon, respectively. This variation might be due to the change in nutritional content or might be the change biochemicals compounds in the different malvaceous plants.

The total life cycle of both male and female pink bollworm reared on artificial diet took more days to complete its life cycle than on okra and statistically significant at p=0.05 level of significance. Pink bollworm male took an average of 40.92 ± 4.89 and 39.99 ± 3.53 days, female took 42.28 ± 4.86 and 39.99 ± 3.53 days on artificial diet and okra, respectively. However, average life cycle of pink bollworm was higher on artificial diet (40.92 ± 4.89 days) as compared to okra (38.60 ± 3.60 days). These results are in agreement with Pradhan (2019) and Shrinivas *et al*. (2019), where they recorded the total life cycle of pink bollworm ranged from 40.00-55.00 and 36.50-49.00 days with the mean of 46.82 ± 1.20 and 42.8 ± 4.66 days in *Bt*-cotton. The results are also corroborated with the results reported by Sapna (2014) where they recorded the total life cycle of pink bollworm which took 40.59 ± 4.54 days with the range of 35.00-46.75 days at 30°C in *Bt*-cotton.

Egg hatching, adult emergence and survival rate

There was a significant difference in egg hatching, adult emergence and survival rate of pink bollworm reared on artificial diet and okra. The higher per cent egg hatching (88.70%) was documented from the eggs laid by the adults obtained after feeding on artificial diet as compared to okra fed pink bollworm (84.90%). Similarly, per cent adult emergence and survival rate of pink bollworm fed with artificial diet was highest (89.64 and 87.48%, respectively) as compared to okra fed pink bollworm (81.34 and 74.16%, respectively) (Fig. 1).

Weight of different stages of pink bollworm reared on different food source

Weight of the different stages of pink bollworm reared on artificial diet and okra showed a significant difference at p=0.05 level of significance (except male prepupa). All the different stages of pink bollworm reared on artificial diet recorded higher weight as compared to the pink bollworm reared on okra. Fourth instar larvae, prepupae, pupae and adult moth of female recorded higher average weight as compared to fourth instar larvae, prepupae, pupae and adult moth of male, when reared on artificial diet as compared to okra (Fig. 2).

The fourth instar male recorded an average of 28.39 ± 3.95 and 25.08 ± 3.95 mg, and fourth instar female recorded an average of 30.12 ± 3.05 and 26.72 ± 3.05 mg, when reared on artificial diet and okra respectively. Similarly, Fand *et al*. (2019), Jothi *et al*. (2016), Sapna (2014) and Dharajothi *et al*. (2010) reported 25.00 ± 3.63, 21.40 ± 3.63, 30.12 ± 1.11 and 21.40 ± 3.63 mg, respectively in fully grown larvae of pink bollworm in *Bt*-cotton.

Male prepupal weight was 22.35 ± 2.28 and 21.36 ± 2.11 mg; female prepupal weight was 25.73 ± 2.69 and 22.89 ± 2.23 mg; male pupal weight was 20.20 ± 1.11 and 14.58 ± 1.20 mg, and female pupal weight was 21.18 ± 2.23 and 15.96 ± 1.26 mg, when reared on artificial diet and okra, respectively (Fig. 2). Pradhan (2019), who recorded an average weight of 27.34 ± 2.27 and 29.22 ± 1.13 mg with a range of 22.35-30.12 and 26.86-30.34 mg in male and female pupa of pink bollworm on *Bt*-cotton. Similar results were also found by Sapna (2014), where they reported the pupal weight of male and female as 17 ± 0.07 and 25 ± 1.11 mg, respectively. In addition, Fand *et al*. (2019), Jothi *et al*. (2016), Muralimohan *et al*. (2009) and Dharajothi *et al*. (2010) also reported 19.26 ± 0.78, 18.00 ± 2.73, 18.02 ± 3.78 and 18.0 ± 2.73 mg, respectively in pupa of pink bollworm on *Bt* cotton, which are in agreement with current research results.

The male moth recorded an average weight of 15.80±1.15 and 11.80±1.25 mg with a range of 13.73-18.49 and 10.25-14.25 mg, and female moth recorded an average weight of 17.46±1.16 and 12.92±1.58 mg with arrange of 16.11-19.63 and 10.58-15.37 mg, when reared on artificial diet and okra, respectively. Adkisson *et al*. (1960) recorded the average adult weight of 13.20, 14.90, 16.20 and 15.10 mg when reared in bolls, 5 per cent cotton seed meal, 1 per cent cotton seed meal and wheat germ medium, respectively and these results are in partial accordance with the current experiment results

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Author contribution statement

RS and CBNV designed and carried out the experiments, recorded the data interpreted the results, and wrote the manuscript. PYG analyzed the data. All the authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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