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Biology of Pink Bollworm *Pectinophora gossypiella* (Saunders) (Gelechiidae: Lepidoptera) under Laboratory Conditions

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ABSTRACT

The present study on biology of pink bollworm on two modified artificial diets separately was carried out in the Toxicology laboratory, Department of Agriculture Entomology, Post Graduate Institute, Dr PDKV, Akola during October to February 2018–19 and 2019–20 with controlled conditions such as 25±2°C temperature 65±5% RH and photoperiod of 14:10 h light: dark. Two modified artificial diets were prepared separately with some modifications in the ingredients, pink bollworm larvae are reared on two above said modified artificial diets and observations were recorded on different biological parameters like egg incubation period (days), larval period (days), pupal period (days), adult emergence (%), adult male and female longevity(days), preoviposition period (days), oviposition (days), fecundity(no of eggs per female), total life cycle(days) and on morphometry of different stages pink bollworm like egg, first, second, third and fourth instar larval stages, male and female pupa and male and female adult moth. Our results indicated that pink bollworm has four larval instars with mean larval duration of 18.60±0.54 days in modified artificial diet 1 and 17.72±0.36 days in modified artificial diet 2 population respectively. Total life cycle from egg to adult was 46.74±0.31 and 54.71±0.62 days in modified artificial diet 1 and modified artificial diet 2 population respectively. Total life cycle of pink bollworm was completed somewhat earlier in in modified artificial diet 1 in comparison to modified artificial diet 2. Our results showed that there was only a little difference in the biology of pink bollworm on both modified artificial diets, and hence both modified artificial diets can be used for laboratory rearing purposes.

KEYWORDS: Biology morphometry, modified artificial diet, PBW, *Pectinophora gossypiella*

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the economically and socially important cash crops in the world and raw material for the textile industry and it plays a key role in the socio-economic and political affairs of the country and is unanimously known as "White gold" and "King of fibers" (Dahake et al., 2017). Globally cotton is commercially cultivated in more than 82 countries on 33.6 mha area with 25.92 mt of production (Anonymous, 2019). In terms of area under cotton cultivation during 2019–20, India holds premier stake worldwide (36%) followed by the USA (14%) and China (10%) with the productivity of 472 kg ha^{-1} whereas worldwide productivity is 771 kg ha^{-1} (Anonymous, 2020). For a long time (nearly ten years), *Bt* cotton was able to shield the crop from bollworms, reducing pesticide use from 46% to less than 21% (Kranthi, 2012). Pink bollworm has been more aggressive in the last three to four years since it has developed insecticide resistance (Li et al., 1997, Sabry and Nahed, 2013) and to Cry toxin of *Bt* cotton (Tabashink et al., 2004, Dhurua and Gujar, 2011, Ojha et al., 2014, Mohan et al., 2016, Kranthi, 2016).

Pectinophora gossypiella is a significant lepidopteran pest that has spread throughout the cotton-growing countries of world. Pink bollworm is known to cause a 2.8–61.9% (Wan et al., 2004) loss in seed cotton yield as well as a 2.1–7.1% loss in oil yield (Patil, 2003). Saunders described the pink bollworm for the first time in 1843, based on specimens collected from (Broach of Gujarat) India in 1842 and named it *Depressaria gossypiella (*Ali et al., 2015, Sorenson et al., 2012, Cohen, 2001). Later, the name was changed to *Pectinophora gossypiella* (Saunders) (Hunter, 1918). It has become one of the most damaging cotton pests in many of the world's most important cotton-growing regions (EI-Lissy et al., 2005, Fareen et al., 2021, Nawaz et al., 2020).

Pink bollworm, *P. gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is now an endemic pest of *Bt* cotton in the central as well as south zones of India and distributed all over the world (Chen et al., 2020, Islam et al., 2020, Arif et al., 2017). Eggs are laid on all tender parts of the plant especially on bracts. After hatching, PBW larvae are found to infest flowers, feeding on the anthers, pollens by living in a sort of web. Such flowers are characteristically twisted in the form of rosette. Later, larvae bore in to the bolls, burrow through the lint penetrating deep into the immature seeds. When one seed is destroyed, larvae make the tunnel through the developing lint and migrate to another seed and similarly in to the locules. "Double seeds" are formed due to joining of adjacent seeds in the boll while feeding (Fand et al., 2020. Sabry et al., Wu et al., 2008). Biology of the pink bollworm on artificial medium contributes to the understanding of various developmental stages, survival, fecundity and behaviour, which has functional applications in management as well as experience on mass rearing on artificial medium for future basic studies. In considering the above the present study has been planned accordingly.

2. MATERIALS AND METHODS

2.1. Modified artificial diets used

The studies were made in the Toxicology laboratory, Department of Agriculture Entomology, Dr PDKV, Akola, Maharashtra, India during October to February 2018–19 and 2019–20 (20.7002° N, 77.0082° E (latitude and longitude)), to study the biology of PBW on two artificial diets seperately with controlled conditions such as 25±2 °C temperature 65±5% RH and photoperiod of 14:10 h light: dark and the methodology of same are presented in detail below. Modifications were done in quantity of ingredients as described by Dharajothi et al. (2016) and Muralimohan et al. (2009). The details of modified artificial diet 1 and modified artificial diet 2 are provided in Table 1. Additional ingredients like ciprofloxacin and polybion are added in the diet by referring to different authors. The pink bollworm culture was started by collecting infested flowers/green bolls from the Cotton Research Station, Dr. PDKV, Akola. Pink bollworm larvae collected (all instars, majorly survived were later instars) from infested fruiting bodies were used for rearing. Modified artificial diets as mentioned above were prepared for rearing pink bollworm larvae and the diet was poured in the deep well of the rearing tray and the collected larvae were released individually until pupa formation. With the help of a fine hair brush, the pupae were gently removed from the cavity well and placed in a plastic tube (4.0×6.0 cm^2) with a perforated lid until adult emergence After sex differentiation, the adults were paired and newly emerged male and female moths were released in pairs in the oviposition cage to mate. To provide oviposition site for females, a 45-day-old cotton plant pot was kept in an oviposition cage. To create darkness, the oviposition cage was draped in black muslin cloth. Cotton swabs dipped in 5% honey solution were placed in oviposition cages as food for male and female moths. The eggs were collected from the leaves and shoots with a camel hair brush and transferred individually to leaf discs for studying morphology and hatching

After pupation pupae were sexed based on pupal characteristics, such as the position of genital and anal openings, which are located mid-ventrally on the 9^{th} and 10^{th} abdominal segments in males and females, respectively. The distance between the genital pore and the anal pore provides a good character for sexes separation. In the case of females, this distance is more than double that of males (Dharajothi et al., 2016). Such sexed pupae were kept in45×45×60m^3 emergence cages for adult emergence. Ten mating pairs of

Table 1: Composition of modified artificial diet (1) modified artificial diet (2) and for *Pectinophora gossypiella*

Composition of modified artificial diet (1) modified artificial diet (2) and for *Pectinophora gossypiella*			
Modified artificial diet (1)*		Modified artificial diet (2)**	
Ingredients	Quantity (500 ml)	Ingredients	Quantity (500 ml)
Non Bt cotton seed flour	25 g	Non Bt cotton seed flour	25 g
Kabuli chickpea flour	25 g	Kabuli chickpea flour	30 g
Distilled water	300 ml	Soybean flour	20 g
Agar-Agar	17 g	Wheat germ	50 g
Distilled water	100 ml	Distilled water	300 ml
Methyl parahydroxy benzoate	0.5 g	Agar-Agar	17 g
Sorbic acid	0.5 g	Distilled water	100 ml
Streptomycin sulphate	0.5 g	Methyl parahydroxy benzoate	0.5 g
Ciprofloxacin	3 ml	Sorbic acid	0.5 g
L-Cystein	0.1 g	Streptomycin sulphate	0.5 g
Wesson salt mixture	3 g	Ciprofloxacin	3 ml
Polybion	6 ml	L-Cystein	0.1 g
Casein	5 g	Wesson salt mixture	3.5 g
Cholesterol	5 g	Polybion	6 ml
L-Ascorbic acid	2 g	Casein	5 g
Carbendazim	2.5 g	Cholesterol	5 g
Yeast extract	10 g	L-Ascorbic acid	2 g

*Modified from Dharajothi et al. (2016); **Modified from Muralimohan et al. (2009)

adult Pink bollworms were released into an oviposition jar (transparent plastic container 28 cm height 24 cm diameter) for mating. To facilitate feeding of adult moths a cotton swab dipped in 10% honey solution was hung by a thread in the oviposition jar and fresh food was provided daily by changing the swab. A small healthy non-*Bt* cotton twig bearing a few leaves and squares was kept in a plastic vial 4 cm diameter and 5 cm height containing sucrose solution through a hole in the cap, which served as a substratum for adults to rest and oviposit. The oviposition jar's opening was covered with a black cotton cloth that was fastened with rubber bands. After 7 days the cotton twigs were removed from the mating jar and cut open into pieces before being placed in a plastic jar 10 cm diameter and 20 cm height closed its mouth with a black cotton cloth fastened with a rubber bands and inverted inside the growth chamber for incubation. Neonate larvae crawling in the plastic jar were then collected with a camel brush and reared in Eppendorf tubes 1.5 ml containing cotton seed diet and placed in a BOD chamber at 25±1°C and 65±5% RH to study the biology. The observations made on each of the biological stages are detailed below.

2.2. *Fecundity*

Fresh non-*Bt* cotton twigs were dipped in a vial 4 cm diameter and 5 cm height of sucrose solution and ten pairs of adult moths were released to count the total number of eggs laid by an individual female pink bollworm. The twig was replaced daily and the number of eggs laid on the old twig was recorded.

2.3. *Egg*

The colour and morphology of the egg were determined using a Stereo Trinocular microscope Olympus-SZ (16) equipped with a Brand Catcam-130 camera with software power Scope photo for measuring the size of the egg. The eggs were observed twice a day (8 a.m. and 4 p.m.) until the larvae emerged and the incubation period was recorded. The incubation period was defined as the time between the egg being laid and the emergence of the first instar larva.

2.4. *Larva*

The newly emerged larvae were placed individually on artificial diet multi-cavity bock in individual deep well plate with the help of fine camel hair brush to determine the number and duration of different larval instars and total larval period. Diet is changed every three days and all larvae were observed daily for instar changes. The presence of casted head capsules and occasionally exuviae in the rearing wells confirmed an instar change. The duration of each instar was calculated and the length and breadth of each instar were measured. The larval period was defined as the time from hatching to pre-pupation. The duration of each instar was determined by examining the casted skin under a microscope.

2.5. *Pupa*

To record the pupal period, the larvae were observed from the time it stopped feeding and became sluggish to the time it formed a silken cocoon around its body during pupation. The pupal period was measured from the time the larva emerged from the cocoon to the time the adult emerged. Pupae's colour and morphology were also documented. Pink bollworm pupae were kept in the emergence cage until adult eclosion. The pupal period was defined as the time between pre-pupation and adult eclosion.

2.6. Adult

The emerged moths were evaluated based on their colour and size. Five pairs of male and female moths of the same age group were separated in an oviposition cage to study their oviposition periods, adult longevity and fecundity. The length and breadth of their wings, both expanded and not, were also measured using a Vernier Caliper. The sex ratio was calculated using male and female moths from the general culture kept in the laboratory. The newly emerged moths in the cage were fed a 10% honey solution. Adult longevity was defined as the time it took for a person to live from the time they were born until they died.

2.7. Pre-oviposition

Pre-oviposition period was defined as the time between the release of mating pairs of pink bollworm into the mating cage and the oviposition of the first egg.

2.8. Oviposition

The oviposition period was defined as the time between the oviposition of the first egg and the oviposition of the last egg.

2.9. Longevity

The records of deaths of the above males and females were kept separately and the longevity was calculated from the date of emergence to the death of the adult.

2.10. Total life cycle

The total life cycle was defined as the time between the date of egg laying and the date of adult death.

3. RESULTS AND DISCUSSION

3.1. Biology

The pooled results on effect of modified artificial diets on biological parameters of PBW for seasons 2018–19 and 2019–20 have been depicted in Table 3.

3.2. Egg period (days)

Eggs were white when laid but turned yellowish and finally orange red before hatching and were flattened oval sculptured with longitudinal lines that were laid in axils of petioles, underside of young leaves, underside of old leaves at vein junctions, or on squares and flowers Pooled analysis of results reveals that the incubation period of *P. gossypiella* eggs differed significantly between modified artificial diet 1 and modified artificial diet 2 populations with mean of 3.32±0.784 and 3.18±0.468 days in both seasons.

Dharajothi et al. (2016) discovered that incubation period of PBW is 4.8±0.632 days on artificial medium. Similarly, the current findings differ slightly from those of Vennila et al. (2007) who reported that incubation of PBW is 3 days at 35±1°C and 6 days at 27±1°C and El-Sayed (1960) who reported that incubation of PBW is 7.37 days at 25±1°C. This might be due to differences in rearing conditions and food sources.

3.3. Total larval period

The first instar larvae had a pale brown head and a whitish body when they hatched and the neonates were very active moving to the top of the container and actively feeding on artificial diet by entering it. Second instar larvae were creamy white with a prominent dark brown head and dark spots on the dorsal side of the body. Third instar larvae are glossy white with a pink transverse dorsal band interpreted by pale medium and lateral streaks per body segment. The fourth instar larvae were pink with a dark brown head and pinkish bands on the body segments. Total larval period of *P. gossypiella* did not differ significantly between modified artificial diet populations in two successive years 2018–19 and 2019–20 with mean of 20.27±2.86 and 17.72±1.94 days (Table 2).

The current findings are consistent with Muralimohan et al. (2009) observed shortest larval period of 21.34±2.61 days on two phase diet (cotton seed flour + okra). Accordingly, Dharajothi et al. (2016) who observed it to be 25.10±0.994 days when reared on artificial medium. This might be due to nutrition and environment (temperature and humidity).

3.4. Pupal period

Pupation occured in the vial after the completion of the pre pupal period, pupae were brown at first, but later turned dark brown to black at the time of adult emergence and the pupae were oval with a pointed tip. Male pupa were smaller in size and the distance between segments on the ventral side was shorter. Female pupa appear to be larger than male pupa and the distance between segments on the ventral side is greater. Pupal period of *P. gossypiella* differed significantly with mean of 8.27±0.48 and 8.46±0.58 between two modified artificial diet populations populations in two succeeding years 2018–19 and 2019–20.

The current findings on the duration of pre-pupae and pupae were in contrast to previous reports as Henneberry and Clayton (1986) reported a 3.5-day delay in pre-pupal and pupal development at fluctuating temperatures ranging from 18.3 to 35°C compared to development at constant temperatures of 26°C. Muralimohan et al. (2009) discovered that when reared on a two phase diet (cotton seed flour and okra) the pupal period was the shortest with 7.96±1.37 days. When reared on artificial medium Dharajothi et al. (2016) reported pupal periods of 7.9±0.88 days.

The variation could be attributed to the lack of differentiation of pre-pupae and pupae in the life cycle, the fulfilment of nutritional requirements in larval stages, effective substrate to pupate and the environment. The state of diapause in the larval or pre-pupal stage was influenced by hormones and

Table 2: Effect of modified artificial diets on biological parameters of pink bollworm (2018–19 and 2019–20 pooled)

Biological parameters	Modified artificial diet 1*	Modified artificial diet 2**	Variance	t-test
	Mean ±SD	Mean ±SD		
Egg incubation period (days)	3.32±0.784	3.18±0.568	0.185	0.027^{S}
Larval period (days)	20.27±2.86	17.72±1.94	2.636	0.236^{NS}
Pupal Period (days)	8.27±0.48	8.46±0.58	3.844	0.021^{S}
Adult emergence (%)	89.37±9.73	91.36±10.48	105.63	0.023^{S}
Adult male longevity (days)	8.825±0.48	7.34±0.37	0.326	0.066^{NS}
Adult female longevity (days)	9.82±0.51	9.184±0.50	0.178	0.016^{S}
Preoviposition period (days)	7.893±0.49	8.435±0.54	0.158	0.013^{S}
Oviposition (days)	2.57±0.53	2.04±0.51	0.150	0.013^{S}
Fecundity (No. of eggs per female)	123.84±13.53	132.74±14.82	160.23	0.025^{S}
Total Life cycle (days)	42.82±5.82	51.544±6.62	6.82	0.019S

*S: "P" value non significant at 1%; *NS: "P" value non significant at 1%; *Modified from Dharajothi et al. (2016); **Modified from Muralimohan et al. (2009)

the temperature at the time. Several workers have reported the diapausing state in the larval stage in cooler climates. According to Metcalf and Metcalf (1993) the pink bollworm can remain dormant for up to 2.5 years. According to Michel and Gomez (1992) almost all pink bollworm larvae in Paraguay enter diapause from April to November.

3.5. Adult longevity

Adults were small moths with dark brown forewings with irregular black markings and silvery grey hindwings with no distinct markings. Both wings were elongated, fringed with long hairs posteriorly and the tip of the hind wing was sharply pointed. Adult female longevity of *P. gossypiella* varied significantly between artificial diet populations with a mean of 9.82±0.51 days and 9.184±0.50 in research period of 2018–19 and 2019–20. These differences in adult longevity of pink bollworm may be due to the use of natural diets such as green bolls and squares in previous studies, as opposed to the current study, which used a semisynthetic diet.

3.6. Pre-oviposition period

The pre-oviposition period of *P. gossypiella* showed significant variation in both modified artificial diet populations with a mean of 7.893±0.49 and 8.435±0.49 days. The current results of the pre-oviposition period of the pink bollworm are in complete agreement with the findings of Dharajothi et al. (2016) who found that the pre-oviposition period of the pink bollworm ranged from 8 to 10 days. Similarly, Muralimohan et al. (2009) reported that the pink bollworm pre oviposition period was 8.00±1.54 days which was consistent with the current findings.

3.7. Oviposition period

The oviposition period was 2.79±0.15 and 2.29±0.29 days in the modified artificial diet 1 and modified artificial diet 2 populations of the year 2018–19 with a range of 2.1–2.90 and 1.6–2.7 days respectively indicating no significant difference. The oviposition period in 2019–20 population was 2.5±0.23 and 2.01±0.46 days in the modified artificial diet 1 and modified artificial diet 2 populations with a range of 2.0–2.5 and 1.8–2.4 days respectively which show a significant difference with each other.

The current results of the pre-oviposition period of the pink bollworm are in complete agreement with the findings of Muralimohan et al. (2009) who reported that the pink bollworm oviposition period was 2.0–2.5 days which was consistent with the current findings.

3.8. Fecundity

The average number of eggs laid by pink bollworm (PBW) females in modified artificial diet 1 population was 123.84±13.53 eggs and 132.74±14.82 eggs in modified artificial diet 2 population with a significant difference.

The current findings are consistent with the findings of Malthankar et al. (2014) who reported PBW female fecundity of 110.6 eggs per individual. Adkinson et al. (1960) reported similar results, reporting 98.1 eggs from moths reared on cotton square. Whereas he also recorded 204.3 eggs from moths reared on cotton bolls and 312.2 eggs from wheat germ diet which contradicts the current findings, the variation in fecundity of pink bollworm is due to diet constituents which have a large impact on the reproductive stimuli of pink bollworm.

3.9. Total life cycle

The total life cycle of PBW population on modified artificial diet 1 population was completed significantly faster in

42.82±5.82 days than in the modified artificial diet 2 population in 51.54±6.62 days with a significant difference. The findings are comparable to those of Malthankar et al. (2014) who discovered that the total life cycle of the pink bollworm averaged 52.31 days.

3.10. Morphometry of modified artificial diet populations of pink bollworm

The pooled results on effect of modified artificial diets on morphometry of PBW for seasons 2018–19 and 2019–20 have been depicted in Table 3.

3.10.1. Egg

The average length and breadth of eggs of PBW when reared on two modified artificial diets showed no significant variation in both consecutive years 2018–19 and 2019–20 with mean of 0.435±0.02:0.437±0.01 and 0.1903±0.01:0.246±0.03. The current findings are comparable to those of Vennila et al. (2017) who reported that the length and breadth of freshly laid eggs ranged between 0.49±0.04 and 0.27±0.04 mm respectively.

3.10.2. First instar larva

Pooled review of data reveals that the average length and breadth of first instar larva of PBW when reared on two modified artificial diets shows no significant variation with a mean of 0.492±0.01 : 0.512±0.02 in modified artificial diet 1 and 0.14±0.02 : 0.17±0.04 in modified artificial diet 2 in both consecutive years.

Dharajothi et al. (2016) reported the length and breadth of first instar larvae fed on *Bt* cotton bolls as 0.54±0.01 mm and 0.17±0.02 mm which are consistent with the current findings.

3.10.3. Second instar larva

Pooled review of data reveals that the average length and breadth of second instar larva of PBW when reared on two modified artificial diet showed no significant variation with a mean of 0.934±0.02 : 0.879±0.01 in modified artificial diet 1 and 0.181±0.05:0.17±0.04 in modified artificial diet 2 in both consecutive years in both consecutive years 2018–19 and 2019–20.

The current findings are consistent with those of Muralimohan et al. (2009) who discovered that second instar larvae were 0.91±0.02 mm in length and 0.18±0.005 mm in breadth. Malthankar et al. (2014) found that second instar larvae ranged in size from 1.02±0.12 mm in length to 0.25±0.01 mm in breadth which is comparable to the current findings.

3.10.4. Third instar larva

The average length and breadth of third instar larva of PBW when reared on two modified artificial diet showed no significant variation in both consecutive years 2018–19 and 2019–20.

The current findings are in concurrent with Malthankar et al. (2014) stated that third instar larvae are 6.96±0.65 mm

Table 3: Effect of modified artificial diets on morphometry of different stages of pink bollworm (2018-19 & 2019-20 pooled)

Biological parameters	Length(mm) (2018-19 and 2019-20 pooled)		Breadth (mm) (2018-19 and 2019-20 pooled)		Length(mm) (2018-19 and 2019-20 pooled)		Breadth (mm) (2018-19 and 2019-20 pooled)	
	Modified artificial diet 1*	Modified artificial diet 2**	Modified artificial diet 1*	Modified artificial diet 2**				
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Variance	t-test	Variance	t-test
Egg	0.435±0.02	0.437±0.01	0.1903±0.01	0.246±0.03	0.034	1.274^{NS}	0.021	0.326^{NS}
Larval stages								
I instar	0.492±0.01	0.512±0.02	0.14±0.02	0.179±0.04	0.0340	0.806^{NS}	1.837	0.522^{NS}
II instar	0.934±0.02	0.879±0.01	0.181±0.05	0.17±0.04	1.50	0.654^{NS}	2.13	0.726^{NS}
III instar	5.63±0.29	5.42±0.26	0.164±0.03	0.172±0.04	0.316	1.812^{NS}	0.624	0.323^{NS}
IV instar	8.92±1.65	9.97±1.75	0.52±0.36	0.496±0.032	5.726	0.048^{S}	0.4026	0.088^{NS}
Pupa								
Male	4.253±0.13	4.273±0.11	1.29±0.04	1.311±0.06	0.0648	1.836^{S}	0.0174	2.672^{S}
Female	4.52±0.17	4.57±0.8	1.272±0.13	1.414±0.18	0.016	0.042^{S}	0.0171	0.028^{S}
Adult								
Male	3.41±0.09	3.371±0.13	1.79±0.10	1.803±0.13	0.0856	0.05^{NS}	0.1932	0.059^{NS}
Female	4.554±1.14	4.58±1.23	1.293±0.09	1.79±0.15	0.0891	0.039^{S}	0.0769	0.048^{S}

*S: "P" value significant at 1%; *NS: "P" value non significant at 1% *Modified from Dharajothi et al. (2016); **Modified from Muralimohan et al. (2009)

in length and 1.62±0.27 mm in breadth which differs from the current findings. The current findings are consistent with those of Dharajothi et al. (2016) who found that the size of third instar larvae ranged from 5.38±0.29 mm in length to 0.16±0.03 mm in breadth.

3.10.5. Fourth instar larva

Pooled analysis of data reveals that the average length and breadth of fourth instar larva of PBW when reared on two modified artificial diet showed significant variation in length and no significant variation in breadth in both consecutive years 2018–19 and 2019–20.

The findings are consistent with those of Muralimohan et al. (2009) who reported that the length and breadth of fourth instar larvae were 9.16±0.08 and 0.45±0.19 mm respectively. Similarly, the current findings agree with Malthankar et al. (2014) who determined the length and breadth of fourth instar larvae to be 9.14±1.65 and 0.46±0.36 mm respectively.

3.10.6. Pupa

3.10.6.1. Male

Pooled review of data reveals that the average length and breadth of male pupa of PBW when reared on two modified artificial diet showed significant variation with a mean of 4.253±0.13:4.273±0.11 in modified artificial diet1 and 1.29±0.04:1.311±0.06 in modified artificial diet 2 in both consecutive years .

3.10.6.2. Female

The average length and breadth of female pupa of PBW when reared on two modified artificial diet showed significant variation with a mean of 4.52±0.17:4.57±0.8 in modified artificial diet1 and 1.272±0.13:1.414±0.18 in modified artificial diet 2 in both consecutive years.

The findings are consistent with those of Malthankar et al. (2014) who reported that the length and breadth of the pupa were 5.05±0.43 and 1.96±0.24 mm respectively. Similarly, the current findings are consistent with Dharajothi et al. (2016) reported the length and breadth of male PBW pupa as 4.22±0.13 and 1.34±0.12 mm and the length and breadth of female PBW pupa as 4.63±0.05 and 1.24±0.11 mm respectively.

3.10.7. *Adult*

3.10.7.1. Male

The length and breadth of male adult of PBW when reared on two modified artificial diet showed no significant variation with a mean of 3.41±0.09 : 3.371±0.13 in modified artificial diet 1 and 1.79±0.10 : 1.803±0.13 in modified artificial diet 2 in both consecutive years.

3.10.7.2. Female

The average length and breadth of male adult of PBW when reared on two modified artificial diets show no significant variation with a mean of 4.554±1.14:4.58±0.8 in modified artificial diet1 and 1.272±0.13:1.414±0.18 in modified artificial diet 2 in both consecutive years.

The findings are consistent with those of Muralimohan et al. (2009) who reported that the length and breadth of the pupa were 4.37±1.20 and 1.60±0.09 mm respectively.

4. CONCLUSION

Two Modified artificial diets deliver new insights for laboratory rearing of pink bollworm which is quite difficult.

5. REFERENCES

Adkisson, P.L., Vanderzant, E.S., Bull, D.L., Allison, W.E., 1960. A Wheat germ medium for rearing the pink bollworm. Journal of Economic Entomology 53(5), 759–762.

Ali, H., Qasim, M., Saqib, H.S., Arif, M., Islam, S.U., 2015. Synergetic effects of various plant extracts as bio-pesticide against wheat aphid (*Diurophousnoxia* L.)(Hemiptera: Aphididae). African Journal Of Agriculture Science And Technology 3(7), 310–5.

Anonymous, 2020. Global cotton consumption expected to grow in 2020/21. Press release, Secretariat, ICAC. Available at http://www.icac.org/press-release/2020-I/PR5-2018-Global-Cotton-Consumption-Expected-to-Gr.

Anonymous, 2019. Global cotton production to increase in 2019/20. Press release, Secretariat, ICAC. Available at http://www.icac.org/pressRelease/2017/PR-41-17.

Arif, M., Siddique, A.M., Farooq, M., Ali, H., Ul, Islam. S., Asad, M., 2017. Spatio-temporal distribution of the peach fruit fly, Bactrocerazonata (Diptera: Tephritidae) infesting citrus orchards at Sargodha, Pakistan. Acta Entomologist Sindh 60(12), 1457–1466.

Chen, Z., Wang, G., Li, M., Peng, Z., Ali, H., Xu, L.,2020. Development of single nucleotide polymorphism (snp) markers for analysis of population structure and invasion pathway in the coconut leaf beetle *Brontispa longissima* (Gestro) using restriction site-associated DNA (RAD) genotyping in Southern China. Insects 11(4), 230.

Cohen, A.C., 2001. Formalizing insect rearing and artificial diet technology. American Entomology 47 (4),198–206.

Dahake, A.B., Patil, P.G., 2017. Production of particle boards from cotton stalks-an eco-friendly way of bio- mass utilization. Agriculture Engineering Today 41(3), 32–35.

Dharajothi, B., Naik, V.C.B., Kranthi, S., Kranthi, K.R.,

Valarmathi, R., 2016. Viable mass production method for cotton pink bollworm, *P. gossypiella* (Saunder). Journal of Basic and Applied Zoology 73(1), 9–12.

Dhurua, S., Gujar, G.T., 2011. Field-evolved resistance to Bttoxin Cry1Ac in the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), from India. Pest Management Science 67(8), 898–903

El-Sayed, M.T., Rahman, H.A., 1960. On the biology and life history of the pink bollworm, *Pectinophora gossypiella* (Saunders). Bulletins Society. Entomology. Egypt. (XLIV), 71–90.

Fand, B.B., Nagrare, V.S., Deshmukh, V., Naikwadi, B.V., Gokte, N. N., Waghmare, V.N., 2020. A simple and low-cost laboratory rearing technique for pink bollworm, *Pectinophora gossypiella* (Suanders) (Lepidop- tera: Gelechidae) using detached green bolls of cotton. Phytoparasitica 48(1), 25–33.

Fareen, G.E., Bodlah, I., Bodlah, M.A., Rasheed, M.T., Ali, H., Asif, M. 2021.Colour and Distributional Pattern of Callaspidianotata (Hymenoptera: Figitidae: Aspicerinae) from Pakistan. Pakisthan Journal of Zoology 53(3), 1153.

Henneberry, T.J., Clayton, T.E., 1986. Pink bollworm: prepupal and pupal development and adult emergence patterns as affected by soil temperature and moisture. The South Western Entomologist 2, 101–106.

Islam, W., Noman, A., Akutse, K.S., Qasim, M., Ali, H., Haider, I., 2020. Phyto-derivatives: an efficient eco- friendly way to manage Trogoderma granarium (Everts) (Coleoptera: Dermestidae International Journal of Tropical Insect Science 16, 1–2.

Kranthi, K.R., 2012. *Bt cotton* questions and answers. Indian Society for Cotton Improvement, 1–70

Kranthi, K.R., 2016. Pink bollworm strikes Bt– Cotton. Cotton Statistics and News (Weekly Publication of Cotton Association of India) 35, 1–5.

Liu, Y.C., Wanf, Q.S., Zhang, S.S., Lou, C.X., Ding, S.Y., 1997. Insecticide resistance in field strains of *Pectinophora gossypiella* in china and effects of synergists on deltamethrin and parathion–methyl activity. Pesticide Science 50, 183–186

Malthankar, P.A., Gujjar, G.T., 2014. Dietary influence on the biology and the susceptibility of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) to Cry1Ac toxin from Bacillus thuringiensis. Biopesticide International 10, 152–159

Metcalf, R.L., Metcalf, R.A., 2006. Destructive and useful insects. Their Habits and Control Ed 5, 1104.

Michel, B., Gomez, E., 1992. Diapause of *Pectinophora gossypiella* (Saunders) in Praguay. Cotton et- Fibers Tropicals 47(2), 95–100.

Mohan, K.S., Ravi, K.C., Suresh, P.J, Sumerford, D., Head, G.P., 2016. Field resistance to the Bacillus thuringiensis protein Cry1Ac expressed in Bollgard® hybrid cotton in pink bollworm, *Pectinophora gossypiella* (Saunders) populations in India. Pest Management Science 72, 738–746

Muralimohan, K., Kamath, S., Mohan, K.S., Ravi, K.C., Deeba, G., Sivasupramanian, S., Head, G.P., 2009. Mass rearing diet for the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and its susceptibility to insecticidal Bt proteins. International Journal of Tropical Insect Science 29(2), 102–107.

Nawaz, A., Ali, H., Fiaz, S., Sufyan, M., Qayyum, M.A., Azad, R., 2021. Analysis of food resources, host availability and insecticidal impacts on the fecundity, longevity and parasitism efficiency of Diaertiellarapae (M'intosh). International Journal of Tropical Insect Science 1–4. https://doi.org/10.1007/s42690-021-00616-2 PMID:34518770

Nawaz, A., Gogi, M.D., Naveed, M., Arshad, M., Sufyan, M., Binyameen, M., 2020. In vivo and in vitro assess- ment of Trichoderma species and Bacillus thuringiensis integration to mitigate insect pests of brinjal (*Solanum melongena* L.). Egypt Journal of Biological Pest Control 30, 1–7.

Ojha, A., Sree, K.S., Sachdev, B., Rashmi, M.A., Ravi, K.C., Suresh, P.J., Mohan, K.S., Bhatnagar, R.K., 2014. Analysis of resistance to Cry1Ac in field– collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelichiida) population. GM Crops and Foods 5, 280–286

Sabry, K.H., Nahed, F.A., 2013. Resistance and enzyme assessment of the PBW *Pectinophora gossypiella* (Saunders) to Spinosad. Journal of Animal Plant Science 23, 136–142

Sabry, K.H., Abdou, G.Y., 2016. Biochemical and toxic characterization of some insect growth regulators to the pink bollworm, *Pectinophora gossypiella* (Saunders). American-Eurasian Journal of Sustainable Agriculture 10(1), 8–14.

Sorensen, J.G., Addison, M.F., Terblanche, J.S., 2012. Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. Crop Protection 38, 87–94.

Tabashink, B.E., Liu, Y.B., Carriere, Y., Dennehy, T.J., Morin, S., 2004. Shared genetic basis of resistance to Bt toxin Cry1Ac in independent strains of pink bollworm. Journal of Economic Entomology 97, 721–726

Venilla, S., Biradar, V.K., Sabesh, M., Bambawale, O.M., 2007. Know your cotton insect pests: pink boll- worms. Crop Protection Folder Series 7 of 11, CICR, Kishan

Forum Pvt. Ltd. Available at https://issuu.com/kisanadmin/docs/kycp_pink_final.

Wan, P., Wu, K.M., Huang, M., Wu, J.,2004. Seasonal pattern of infestation by pink bollworm *Pectinophora gossypiella* (Saunders) in field plots of Bt transgenic cotton in the Yangtze River valley of China. Crop Protection 23, 463–467

Wu, J.P., Wu, H.H., Wan, P., Huang, M.S., 2008. Technique for rearing the pink bollworm (*Pectinophora gossypiella*) with artificial diet. Hubei Agriculture Science 47, 7–9.