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COMPARATIVE BIOLOGY AND MORPHOMETRIC STUDIES OF PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) (LEPIDOPTERA: GELECHIIDAE) ON *Bt* AND NON-*Bt* COTTON

Shashank D. U.[1*], Poornima V. Matti[2], S. B. Patil[2] and Manjula S. Maralappanavar[3]
[1] Department of Agricultural Entomology, College of Agriculture,
University of Agricultural Sciences, Dharwad – 580005 (Karnataka), India
[2] Department of Agricultural Entomology, ARS, Dharwad, University of Agricultural Sciences,
Dharwad – 580005 (Karnataka), India
[3] Department of Genetics and Plant Breeding, ARS, Dharwad, University of Agricultural Sciences,
Dharwad – 580005 (Karnataka), India
*Corresponding author E-mail: shankydu98@gmail.com

ABSTRACT

Pink bollworm (PBW) is a prominent pest among cotton bollworms in the context of *Bt* cotton cultivation. Under increased selection pressure PBW evolved resistance to *Bt* toxin which had led to outbreak of PBW in many parts of the country. The present investigation on comparative biology and morphometry was conducted at the Agricultural Research Station (ARS), Dharwad, during 2021-22 in laboratory conditions, with a temperature of 27.90 ± 0.77 °C and relative humidity of 80 ± 4.40 per cent. The average life cycle of PBW was longer in Jadoo BG II (41.11 ± 2.54 days) compared to Jadoo non-*Bt* (38.72 ± 0.46 days). The larval period lasted for 20.51 ± 1.74 and 19.70 ± 0.51 days in Jadoo BG II and Jadoo non-*Bt*, respectively, while the mean pupal period was 7.71 ± 0.36 and 7.50 ± 0.50 in the respective genotypes. Adult emergence rate was 97.96 and 98.21 per cent for Jadoo BG II and Jadoo non-*Bt*, respectively. Adult Females had longer survival rates than males, with Jadoo non-*Bt* exhibiting higher fecundity (120.00 ± 20.00 eggs/ female) than Jadoo BG II (117.00 ± 19.67 eggs/female). Jadoo non- *Bt* cotton displayed higher larval, pupal, and adult weight compared to Jadoo BG II. Furthermore, the morphometric measurements indicated reduced dimensions in larval, prepupal, pupal, and adult stages in *Bt* cotton. Additionally, Jadoo BG II showed higher percentages of deformed pupae (6.25 %) and adults (3.20 %) compared to Jadoo non-*Bt*. The complex biology of the pink bollworm, despite of being influenced by Cry1Ac toxin in *Bt* cotton, poses challenges due to its internal feeding within developing seeds and the impact on life cycle prolongation, with pest pressure intensifying around 90 days as *Cry* toxin levels diminish.

Keywords : *Bt* genotype, biology, Jadoo, non-*Bt* genotype, morphometry, pink bollworm, *Cry* protein

Introduction

Cotton (*Gossypium* spp.) is a prominent global cash crop known for its fiber and seed oil, belongs to the genus *Gossypium* within the Malvaceae family. It is cultivated in over 100 countries across 32 million hectares. In India, several insects and mites have been identified that infest cotton at various phases of development (Agarwal *et al.,* 1984). The pink bollworm (PBW) infestation in non-*Bt* cotton used to cause substantial losses, affecting seed cotton yield, oil content, and boll opening. Cotton infested with a diverse array of insect pests, with up to 130 distinct species, and three types of bollworms have been identified in India, among which the pink bollworm has become a center of significant concern (Ghosh, 2001). This pest has a wide distribution across India and causes considerable damage to cotton output (Dhurua and Gujar, 2011; Naik *et al.,* 2018). The pink bollworm's life cycle encompasses four developmental stages: egg, larva, pupa, and adult. It undergoes four larval instars before pupating, with the total life cycle

taking 45 to 70 days on cotton (Zinzuvadiya *et al.,* 2017). Interestingly, the life cycle duration of the pink bollworm is longer on *Bt* cotton than on non-*Bt* cotton (Shrinivas *et al.,* 2019), and *Cry*1Ac, a *Bt* toxin, has shown positive effects on all stages of the pest's life (Rajput *et al.,* 2019). The pest strikes most during later part of the crop (90 to 120 days after sowing) when the second and third flues are expected. During this stage, there is a generally observed decrease in *Cry* toxin levels within *Bt* cotton plants and these transgenics have indeterminate type of growth that leads to continuous formation of bolls with reduced quantity of toxin, which coincides with PBW incidence (Badiger, 2010). The internal feeding habits of the pink bollworm larvae within the developing cotton bolls make them highly resilient to traditional pesticides (Mohan *et al*., 2016). As a result, farmers often remain unaware of the damage caused by PBW until boll opening, making targeted control measures challenging. To address this, comparative biology and morphometric studies have been undertaken to better understand and manage the challenges posed by the pink bollworm in the context of *Bt* cotton.

Material and Methods

The biology and morphometry of pink bollworm were studied at 130 DAS under laboratory condition having 27.90 ± 0.77 °C temperature, 80 ± 4.40 per cent relative humidity at Agricultural Research Station (ARS), Dharwad from December 2021 to January 2022. Eggs were procured from National Bureau of Agricultural Insect Resource (NBAIR) (Figure 1a). After hatching of eggs, twenty neonates were collected and reared on an artificial diet standardized by Dharajothi *et al.* (2016) incorporated with seed powder of Jadoo BG II and Jadoo non-*Bt*. Ten healthy second instar larvae were selected and reared on natural food consisting of seed bits of respective genotypes. Seed bits were retrieved from green bolls of both genotypes and replicated thrice. One larva per well was maintained during the experiment. A total of 10 wells were used out of 25 wells of the rearing tray. In each well 3 to 4 seed bites were placed and changed every two days to maintain moisture and avoid fungal growth. The larvae (Figure 1a) were reared in the laboratory till the adult emergence. Pupae (Figure 1b) were collected and washed in clean water gently and sexed based on the position of genital and anal openings wherein, the genital and anal pores are situated mid-ventrally on the 9^{th} and 10^{th} in males, 8^{th} and 10^{th} abdominal segments in females. The distance between the genital pore and anal pore affords a good character for the separation of the sexes (Figure 1b). In the case of females, this distance is more than double as compared to males (Dharajothi *et al.,* 2010). Such sexed pupae were transferred to a mating cage for adult emergence. After adult emergence, they were allowed for mating in a mating cage (Figure 2). A small vial with a cotton plug filled with 10% honey solution was kept in the cage as adult food and cotton twig along with cotton lint was stuffed in a small plastic container containing sucrose solution was also placed in the cage for the egg laying purpose. Leaves with deposited eggs were collected and transferred to a plastic container for egg hatching with a lid closed with perforated cloth and tied with a rubber band. Observations on duration of each stage of life cycle were recorded along with deformity in pupal and adult stages. Morphometric measurements of different stages of pink bollworm were taken separately for comparison. Data was recorded by observing under stereo binocular microscope in the Leica application. Observations were taken on measurements *viz.,* the length and width of eggs, each larval instar, male and female pupae, adult male and female, the wing span of both male and female moths and observations on morphological characters were also recorded. The analysis was conducted through the computation of statistical measures, including the mean, standard deviation, and range, for all relevant parameters.

Results and Discussion

The pink bollworm larvae were fed with seed bits of *Bt* and non-*Bt* genotypes, simulating their natural food. When the larvae were in their early stage (first instar), they were not able to eat the seeds effectively due to less developed mandibles. Hence, they were reared on artificial diet incorporated with seed powder of respective genotypes. Healthy second instar larvae were selected and reared on seed bits (natural food) for further biological and morphometric studies. There was difference in duration of life cycle in *Bt* genotype because of effect of Bt toxin which extended total life cycle than non-*Bt* genotype without affecting its fitness with pest pressure intensifying around 90 days as *Cry* toxin levels diminish. But the mechanism and reasons for prolongation of life cycle in *Bt* genotypes in contrast to non-*Bt* genotypes are needed to be studied further.

Larvae

Upon hatching, the mean duration of first instar larvae was 3.39 ± 0.35 days in Jadoo BG II and 3.16 ± 0.29 days in Jadoo non-*Bt*, with ranges of 3.00 - 3.67 and 2.98 - 3.50 days, respectively. Second instar larvae exhibited a mean duration of 4.29 ± 0.50 days in Jadoo BG II and 4.49 ± 0.02 days in Jadoo non-*Bt*, with ranges of 4.00 - 4.86 and 4.46 - 4.50 days, respectively.

During the third instar, larvae were recorded a mean duration of 5.83 ± 0.76 days in Jadoo BG II and 5.34 ± 0.28 days in Jadoo non-*Bt*, with ranges of 5.00 - 6.50 and 5.01 - 5.50 days, respectively. Following the final molt, the duration of the fourth instar ranges from 6.50 - 7.50 days in Jadoo BG II and 6.50 - 7.00 days in Jadoo non-*Bt*, with a mean of 7.00 ± 0.50 days in Jadoo BG II and 6.72 ± 0.26 days in Jadoo non-Bt. The total larval period was longer in Jadoo BG II (20.51 ± 1.74 days) compared to Jadoo non-Bt (19.70 ± 0.51 days) (Table 1). These findings align with previous studies, supporting the observed differences in the total larval period of pink bollworm on *Bt* cotton. Fand *et al.* (2019) and Shah *et al.* (2013) reported the total larval period of pink bollworm was 21.50 ± 1.04 and 21.50 ± 1.04 days on *Bt* cotton. Additionally, Sapna (2014) observed a mean larval period of 23.15 ± 3.23 days at 25 °C, consistent with the results of present study.

Pupae

After four successive moults, each larva in both genotypes progressed through the pre-pupal and pupal stages. Pupation occurred within vials. The mean pre-pupal period before pupation was 1.81 ± 0.54 and 1.45 ± 0.38 days for Jadoo BG II and Jadoo non-*Bt*, respectively, within the range of 1.50 - 2.44 and 1.30 - 1.60 days (Table 1). Pupation rates varied between genotypes, with Jadoo non-*Bt* recording the highest rate at 76.00 per cent, followed by Jadoo BG II at 66.68 per cent. Deformity in the pupal stage was more prevalent in case of the *Bt* genotype. Normal pupae constituted 65.32 per cent (Jadoo BG II) and 74.68 per cent (Jadoo non-*Bt*), while deformed pupae made up 4.00 per cent (Jadoo BG II) and 1.32 per cent (Jadoo non-*Bt*) in the respective genotypes (Table 2). The longest pupal duration occurred in the *Bt* genotype, with a mean of 7.71 ± 0.36 days, ranging from 7.50 to 8.12 days in Jadoo BG II. In comparison, the non-*Bt* genotype showed a comparatively shorter pupal duration of 7.50 ± 0.50 days, ranging from 7.00 to 8.00 days, when reared on the same genotype of non-*Bt* cotton (Table 1). These results are consistent with prior investigations by Zinzuvadiya *et al.* (2017) who found 1.60 ± 0.52 days for male and 1.65 ± 0.49 days of prepupal duration for female pink on *Bt* cotton. Navya (2019) reported the highest pupal duration of 9.82 ± 0.26 days on *Bt* cotton (MRC 7918 BG II) and the shortest, 7.16 ± 0.19 days, on non-*Bt* (Suvin). The current study's findings on pink bollworm pupal and adult malformation align in part with Tanani and Bakr (2018), who noted 66.67 and 25.00 per cent deformed pupae when treated with 1 and 10 ppm of difenolan, respectively.

Adults

The per cent adult emergence of pink bollworm was 97.96 (Jadoo BG II) and 98.21 per cent (Jadoo non-*Bt*), with male longevity of 9.66 ± 0.28 and 8.98 ± 0.53 days, respectively. Whereas the female longevity was 12.50 ± 0.50 (Jadoo BG II) and 11.12 ± 0.33 days (Jadoo non-*Bt*). Deformed adults were higher in *Bt* (3.20%) than non-*Bt* (1.74%) (Table 2). The current findings are in line with Fand *et al.* (2019), who reported 92.32 ± 2.34% emergence on *Bt* cotton, Shrinivas *et al.* (2019), observing 88.32 ± 10.55 and 91.66 ± 8.47 per cent emergence in *Bt*-cotton and okra, and Shrilakshmi (2021), noted 8.55 ± 1.01 and 9.06 ± 0.89 days (*Bt* Everesrt) and 7.96 ± 0.95 and 8.82 ± 0.65 days (non-*Bt* Everest).

Oviposition

The pink bollworm exhibited consistent pre-oviposition, oviposition, and post-oviposition periods across genotypes. Jadoo BG II had slightly longer durations: 3.00 ± 0.50, 2.67 ± 1.04, and 3.88 ± 0.38 days, compared to Jadoo non-*Bt* genotype (2.67 ± 0.76, 2.17 ± 0.58, and 3.50 ± 0.50, respectively) (Table 1). The present results are in corroborated with Pradhan (2019) and Sapna (2014) where they reported the oviposition period of pink bollworm ranged from 1.50 to 2.50 and 2.00 to 2.50 days with a mean of 2.20 ± 0.25 and 2.05 ± 0.53 days on *Bt*-cotton

Eggs

In Jadoo BG II and Jadoo non-Bt, the average fecundity was 117.00 ± 19.67 and 120.00 ± 20.00 eggs per female, with hatching percentages of 62.27 ± 5.91 and 75.95 ± 8.61 per cent (Table 1). Similar findings were observed by Shrilakshmi (2021) in *Bt* and non-*Bt* Everest, with fecundity of 117.40 ± 10.22 and 124.92 ± 15.83 eggs per female, respectively and Shrinivas *et al.* (2019) noted an average fecundity of 118.65 ± 16.81 (range: 100 to 185) eggs per female in okra. Egg hatchability ranged from 55.56 to 66.67 per cent in Jadoo BG II and from 67.86 to 85.00 per cent in Jadoo non-*Bt*, with means of 62.27 ± 5.91 and 75.95 ± 8.61 per cent. These results align with Shrilakshmi (2021), who observed hatchability percentages of 67.99 ± 2.61 and 82.91 ± 3.04 per cent in *Bt* and non-*Bt* Everest, respectively. The incubation period of pink bollworm eggs was slightly longer in the *Bt* genotype (3.95 ± 0.69 days in Jadoo BG II) compared to the non-*Bt* genotype (3.91 ± 0.66 days in Jadoo non-*Bt*), which is consistent with Shrilakshmi (2021), who recorded incubation periods of 4.18 ± 0.30 and 4.41 ± 0.36 days in *Bt* and non-*Bt* Everest, respectively.

Total life cycle

The total life cycle of pink bollworm was longer on *Bt* genotype compared to non-*Bt*, with females having a longer cycle (42.53 ± 2.55 and 39.83 ± 0.77 days in Jadoo BG II and Jadoo non-*Bt*, respectively) than males (39.69 ± 2.55 and 37.69 ± 1.01 days in in Jadoo BG II and Jadoo non-*Bt*, respectively). The average life cycle was 41.11 ± 2.54 days on Jadoo BG II and 38.72 ± 0.46 days on Jadoo non-*Bt* (Table 1). These findings comparable with Shrilakshmi (2021), who reported a total life cycle of 48.46 ± 1.69 and 45.01 ± 2.81 days in *Bt* and non-*Bt* Everest, respectively. Navya (2021) recorded the shortest life cycle of 32.44 ± 0.93 days in DDhc-11 and the longest in MRC-7918 (43.43 ± 1.21 days).

Morphometry of pink bollworm

Distinct morphometric measurements were recorded for various developmental stages of pink bollworm when raised on both *Bt* and non-*Bt* cotton genotypes, enabling a comprehensive comparison. Additional morphological characteristics were also documented, and a detailed presentation of the results (Table 3) follows.

Eggs

Pink bollworm eggs exhibited a color progression from white to yellowish and finally to orange-red before hatching. They were flattened ovals adorned with longitudinal lines. The dimensions of these eggs showed variation: 0.36 - 0.40 mm in length and 0.17 - 0.21 mm in width, with a mean of 0.38 ± 0.02 mm (length) and 0.19 ± 0.02 mm (width) in Jadoo BG II. In Jadoo non-*Bt*, the range was 0.38 - 0.45 mm (length) and 0.19 - 0.29 mm (width), with a mean of 0.41 ± 0.04 mm (length) and 0.23 ± 0.05 mm (width). Eggs from non-*Bt* genotype was larger in both length and width compared to *Bt* genotypes, consistent with Rakhesh (2021) documented PBW egg dimensions on *Bt* cotton, averaging 0.42 ± 0.02 mm (length) and 0.19 ± 0.01 mm (width) within a range of 0.38 - 0.45 mm (length) and 0.18 - 0.21 mm (width). In comparison, eggs from okra exhibited an average length and width of 0.39 ± 0.02 mm and 0.18 ± 0.01 mm, respectively, with a range of 0.35 - 0.43 mm (length) and 0.16 - 0.20 mm (width) (Shrinivas *et al.,* 2019).

Larvae

The first instar pink bollworm larva displayed pale brown head and whitish body, actively moving. In Jadoo BG II, the mean length and width ranged from 0.79 - 0.99 mm and 0.16 - 0.20 mm, with averages of 0.88 ± 0.10 mm and 0.18 ± 0.02 mm. In Jadoo non-*Bt*, length was 0.86 – 1.09 mm and width were 0.29 – 0.31 mm, with averages of 0.98 ± 0.12 mm and 0.30 ± 0.01 mm.

Second instar larvae were creamy white with a dark brown head and dorsal spots. In Jadoo BG II, length and width ranged from 3.19 - 3.24 mm and 0.29 - 0.35 mm, averaging 3.21 ± 0.03 mm and 0.32 ± 0.03 mm. In Jadoo non-*Bt*, length was 3.87 - 4.07 mm and width was 0.38 - 0.61 mm, averaging 3.98 ± 0.10 mm and 0.52 ± 0.12 mm, respectively.

Third instar larvae displayed a glossy white appearance with pink transverse dorsal bands and pale lateral streaks per segment. In Jadoo BG II, length and width ranged from 6.89 - 8.07 mm and 0.67 - 1.30 mm, with averages of 7.57 ± 0.61 mm and 1.02 ± 0.32 mm. In Jadoo non-*Bt*, lengths were 6.99 - 8.73 mm and widths were 0.89 - 1.29 mm, with averages of 7.87 ± 0.87 mm and 1.07 ± 0.20 mm.

Fourth instar larvae were pink with dark brown heads and pinkish bands on segments. In Jadoo BG II, length and width ranged from 10.95 - 11.81 mm and 1.99 - 2.27 mm, with averages of 11.25 ± 0.48 mm and 2.09 ± 0.16 mm, respectively. In Jadoo non-*Bt*, lengths were 10.99 - 11.60 mm and widths were 1.89 - 2.54 mm, with averages of 11.29 ± 0.31 mm and 2.23 ± 0.33 mm.

The current findings on the size of pink bollworm larvae in this experiment align with the research conducted by Nagamandla and Maheswari (2021), who reported an average of 0.92 ± 0.04 and 0.92 ± 0.06 mm of length of First instar larvae with a range of 0.82 - 1.00 and 0.83 - 1.02 mm; 3.44 ± 0.18 and 3.48 ± 0.14 mm of length of second instar larvae with a range of 3.30 - 3.93 and 3.33 - 3.65 mm; 6.84 ± 0.74 and 6.89 ± 0.15 mm of length of third instar larvae with a range of 3.30 - 3.93 and 3.33 - 3.65 mm; 9.53 ± 0.12 and 9.60 ± 0.30 mm of length of fourth instar larvae with a range of 9.32-9.69 and 9.02-9.96 mm on *Bt*-cotton and okra, A degree of agreement is also observed with a study by Rakhesh (2021), where the dimensions of first instar larvae reared on *Bt*-cotton were slightly different, and those reared on *A. ficulneus* showed distinct measurements, who noticed the length and breadth of PBW first instar larva was ranged from 0.83 -1.02 and 0.20 - 0.26 mm with an average of 0.90 ± 0.06 and 0.23 ± 0.02 mm, respectively when reared on *Bt*-cotton. However, length and breadth of first instar larva reared on *A. ficulneus* was ranged from 0.80-0.92 and 0.18-0.21 mm with an average of 0.84 ± 0.04 and 0.20 ± 0.01 mm, respectively. The observed morphometric differences in the larval instars between *Bt* and non-*Bt* genotypes may be attributed to variations in nutritional content or biochemical

compounds of the respective seed bits collected from each genotype (Vijaykumar, 2019).

Pupae

Initially, the pupae displayed a brown color, gradually darkening to black upon adult emergence. These pupae were oval with a pointed tip. Male pupae were smaller with shorter distances between segments on the ventral side. In contrast, female pupae were larger, with greater ventral segment distances than males. In Jadoo BG II, the pre-pupa length and width ranged from 7.92 – 8.76 mm and 2.20 – 2.68 mm, with means of 8.34 ± 0.42 mm and 2.42 ± 0.24 mm, respectively. For Jadoo non-*Bt*, these measurements ranged from 7.67 – 8.49 mm in length and 2.23 – 2.56 mm in width, with means of 8.14 ± 0.42 mm and 2.41 ± 0.17 mm, respectively.

The male pupae in Jadoo BG II had a length between 7.08 – 8.10 mm and width of 2.49 – 2.60 mm, with means of 7.66 ± 0.52 mm and 2.54 ± 0.06 mm, respectively. For Jadoo non-*Bt*, the length range was 7.32 – 8.59 mm, with a mean of 7.83 ± 0.41 mm, and the width was 2.44 ± 0.14 mm. The female pupae in Jadoo BG II had length between 8.28 – 8.60 mm and width of 2.10 – 2.54 mm, with means of 8.43 ± 0.16 mm and 2.35 ± 0.23 mm, respectively. In Jadoo non-*Bt*, the length range was 7.92 – 8.12 mm, with a mean of 4.42 ± 0.19 mm, and the width ranged from 2.17 – 2.65 mm, with a mean of 1.42 ± 0.24 mm.

Non-*Bt* genotype pupae exhibited larger dimensions than *Bt* genotype pupae. The pre-pupa measurements are comparable with Zinzuvadiya *et al.* (2017), who found similar length (7.67 ± 0.35 mm) and breadth (2.51 ± 0.09 mm) in pink bollworm pre-pupae. Nagamandla and Maheswari (2021) reported *Bt*-cotton and okra pre-pupae lengths averaging 8.06 ± 0.09 mm and 8.05 ± 0.10 mm, respectively, with ranges of 7.89 - 8.20 mm and 7.90 - 8.29 mm. For width, they found averages of 2.32 ± 0.14 mm and 2.32 ± 0.16 mm, respectively on *Bt*-cotton and okra.

Adults

The adult pink bollworm moth was small, dark brown, featuring irregular black markings on the forewings, elongated hind wings with a silvery grey color and no distinct markings, and long-fringed with hairs, culminating in a sharply pointed posterior and tip on the hind wing. Morphometric measurements were taken with and without wing expansion. In Jadoo BG II, the adult male (body) had lengths ranging from 6.76 – 6.91 mm and widths of 1.32 – 2.39 mm, with means of 6.85 ± 0.08 mm and 1.98 ± 0.58 mm. In Jadoo non-*Bt*, these measurements ranged from 6.79 – 7.13 mm in length and 1.89 – 2.27 mm in width, with means of 6.92 ± 0.19 mm and 2.04 ± 0.20 mm. Similarly, for adult females (body) in Jadoo BG II, lengths ranged from 6.99 – 7.19 mm and widths from 2.01 – 2.56 mm, with means of 7.06 ± 0.11 mm and 2.34 ± 0.29 mm. In Jadoo non-*Bt*, the lengths ranged from 6.89 – 7.40 mm, and widths from 2.65 – 2.81 mm, with means of 7.18 ± 0.26 mm and 2.73 ± 0.08 mm. Additionally, wing expansion measurements for adult males and females in Jadoo BG II ranged from 14.92 – 17.48 mm and 16.98 – 17.84 mm, with means of 15.88 ± 1.39 mm and 17.36 ± 0.44 mm. In Jadoo non-*Bt*, these measurements ranged from 14.27 – 17.65 mm for males and 16.94 – 18.00 mm for females, with means of 15.97 ± 1.69 mm and 17.40 ± 0.55 mm.

Females are superior than male in terms of length and width across the genotypes but *Bt* genotypes recorded lower measurements of length and width than non-*Bt* genotypes. These findings align with Nagamandla and Maheswari (2021), showing male PBW moth length averaging 5.40 ± 0.28 mm on *Bt*-cotton, 6.65 ± 1.43 mm in female moths. Shah *et al.* (2013) found a 7.00 - 10.00 mm length for both genders on Bt cotton. Similarly, Zinzuvadiya *et al.* (2017) reported 8.37 ± 1.20 mm in males and 8.37 ± 1.12 mm in females, with 17.50 ± 0.57 mm and 16.60 ± 1.09 mm wing spans in males and females, respectively. Regarding wing expansion, our findings match Rakhesh (2021), recording average male and female body breadths of 16.51 ± 0.99 mm and 17.51 ± 0.36 mm, respectively on *Bt*-cotton. However, body breadths with wing expansion on *A. ficulneus* were 16.40 ± 1.01 mm for males and 17.41 ± 0.37 mm for females.

Weight

The presence of *Cry* toxin in the *Bt* genotype led to reduced weight in third and fourth instar larvae, pupae, and adult stages compared to the non-*Bt* genotype (Table 4). Third instar larvae weighed 13.17 ± 0.67 mg (Jadoo BG II) and 14.59 ± 0.64 mg (Jadoo non-*Bt*), while fourth instar larvae weighed 29.39 ± 1.89 mg (Jadoo BG II) and 32.80 ± 2.59 mg (Jadoo non-*Bt*). Pupal weight was 17.56 ± 1.15 mg (male) and 19.82 ± 0.95 mg (female) in Jadoo BG II, and 19.37 ± 1.53 mg (male) and 22.64 ± 1.12 mg (female) in Jadoo non-*Bt*. Adult weight was 15.20 ± 0.91 mg (male) and 16.45 ± 1.25 mg (female) in Jadoo BG II, and 18.26 ± 0.94 mg (male) and 18.34 ± 1.07 mg (female) in Jadoo non-*Bt*. These findings are in line with Shrilakshmi (2021), who reported lower larval (fourth instar) weight of 32.60 ± 2.16 mg in *Bt* cotton and higher weight of 36.69 ± 3.10 mg in non-*Bt* Everest BG II. However, Rakhesh (2021) recorded an average weight

of male and female adults on *A. ficulneus* as 12.92 ± 1.42 mg and 14.66 ± 1.16 mg, respectively.

Conclusion

Pink bollworm presents unique challenges in *Bt* cotton systems due to its internal feeding habits within developing cotton seeds. Our current study on the comparative biology of pink bollworm demonstrates an extended life cycle duration for various life stages in the *Bt* genotype (Jadoo BG II) compared to the non-*Bt* genotype (Jadoo non-*Bt*). Morphometric measurements indicate reduced dimensions in larvae, prepupae, and pupae within the Bt genotypes. The life cycle of pink bollworm, spanning from egg to adult mortality, exhibited differences between hosts, notably longer on *Bt* cotton compared to non-Bt cotton. *Cry*1Ac, a *Bt* toxin, displayed favorable effects on all life stages of the pink bollworm. Pest pressure typically intensifies in the later stages of the crop, around 90 days onwards, where diminishing *Cry* toxin levels in *Bt* cotton contribute to the successful completion of the life cycle.

Table 1 : Comparative duration of life stages of pink bollworm on seed bits of *Bt* and non-*Bt* cotton

Life stages	Duration (days)			
	Jadoo BG II		Jadoo non-*Bt*	
	Mean ± S.D	Range	Mean ± S.D	Range
Egg*				
Incubation period	3.95 ± 0.69	3.50 - 4.75	3.91 ± 0.66	3.40 - 4.65
Larval instars*				
I instar	3.39 ± 0.35	3.00 - 3.67	3.16 ± 0.29	2.98 - 3.50
II instar	4.29 ± 0.50	4.00 - 4.86	4.49 ± 0.02	4.46 - 4.50
III instar	5.83 ± 0.76	5.00 - 6.50	5.34 ± 0.28	5.01 - 5.50
IV instar	7.00 ± 0.50	6.50 - 7.50	6.72 ± 0.26	6.50 - 7.00
Total Larval period	20.51 ± 1.74	18.50 - 21.53	19.70 ± 0.51	19.11 - 20.00
Pupa*				
Pre pupa	1.81 ± 0.54	1.50 - 2.44	1.45 ± 0.38	1.30 - 1.60
Pupa	7.71 ± 0.36	7.50 - 8.12	7.50 ± 0.50	7.00 - 8.00
Adult longevity				
Female*	12.50 ± 0.50	12.00 - 13.00	11.12 ± 0.33	10.87 - 11.50
Male*	9.66 ± 0.28	9.50 - 9.98	8.98 ± 0.53	8.45 - 9.50
Preoviposition period (days)*	3.00 ± 0.50	2.50 - 3.50	2.67 ± 0.76	2.00 - 3.50
Oviposition period (days)*	2.67 ± 1.04	1.50 - 3.50	2.17 ± 0.58	1.50 - 2.50
Postoviposition period (days)*	3.88 ± 0.38	3.50 - 4.25	3.50 ± 0.50	3.00 - 4.00
Fecundity (Eggs/female)**	117.00 ± 19.67	96.00-135.00	120.00 ± 20.00	100.00-140.00
Hatching (%)***	62.27 ± 5.91	55.56-66.67	75.95 ± 8.61	67.86-85.00
Total lifecycle*				
Female	42.53 ± 2.55	40.00 - 45.09	39.83 ± 0.77	38.98 - 40.50
Male	39.69 ± 2.55	37.00 - 42.07	37.69 ± 1.01	36.56 - 38.50
Average Life Cycle	41.11 ± 2.54	38.50 - 43.58	38.76 ± 0.89	37.77 - 39.50

*Mean of 10 individuals (n=10) **Mean of 5 layings (n=5) ***Mean of 10 females (n=10)

Table 2 : Pupation (%) and adult emergence (%) of pink bollworm on seed bits (natural food) of different *Bt* and non-*Bt* cotton of Jadoo

Particulars	Jadoo BG II	Jadoo non-*Bt*
Pupation*		
Pupation (%)	66.68	76.00
Normal Pupa (%)	65.32	74.68
Deformed Pupa (%)	4.00	1.32
Adult emergence*		
Adult emergence (%)	97.96	98.21
Normal Adult (%)	94.48	96.47
Deformed Adult (%)	3.45	1.74

*Mean of individuals of 3 replications

Table 3 : Morphometry of of pink bollworm life stages on seed bits (natural food) of different *Bt* and non-*Bt* cotton genotype

Life stages	Jadoo BG II		Jadoo non-*Bt*	
	Length(mm)	Width(mm)	Length(mm)	Width(mm)
Egg	0.38 ± 0.02 (0.36 - 0.40)	0.19 ± 0.02 (0.17 - 0.21)	0.41 ± 0.04 (0.38 - 0.45)	0.23 ± 0.05 (0.19 - 0.29)
Larva*				
I instar	0.88 ± 0.10 (0.79 - 0.99)	0.23 ± 0.02 (0.16 - 0.20)	0.98 ± 0.12 (0.86 - 1.09)	0.30 ± 0.01 (0.29 - 0.31)
II instar	3.21 ± 0.03 (3.19 - 3.24)	0.32 ± 0.03 (0.29 - 0.35)	3.98 ± 0.10 (3.87 - 4.07)	0.52 ± 0.12 (0.38 - 0.61)
III instar	7.57 ± 0.61 (6.89 - 8.07)	1.02 ± 0.32 (0.67 - 1.30)	7.87 ± 0.87 (6.99 - 8.73)	1.07 ± 0.20 (0.89 - 1.29)
IV instar	11.25 ± 0.48 (10.95 - 11.81)	2.09 ± 0.16 (1.99 - 2.27)	11.29 ± 0.31 (10.99 - 11.60)	2.23 ± 0.33 (1.89 - 2.54)
Pupa*				
Pre pupa	8.34 ± 0.42 (7.92 – 8.76)	2.42 ± 0.24 (2.20 – 2.68)	8.14± 0.42 (7.67 – 8.49)	2.41 ± 0.17 (2.23 – 2.56)
Pupa (Male)	7.66 ± 0.52 (7.08 – 8.10)	2.54 ± 0.06 (2.49 - 2.60)	7.83 ± 0.41 (7.39 – 8.19)	2.44 ± 0.14 (2.32 - 2.59)
Pupa (Female)	8.43 ± 0.16 (8.28 - 8.60)	2.35 ± 0.23 (2.10 - 2.54)	8.02 ± 0.10 (7.92 – 8.12)	2.45 ± 0.25 (2.17 – 2.65)
Adult*				
Adult Male (Body)	6.85 ± 0.08 (6.76 - 6.91)	1.98 ± 0.58 (1.32 - 2.39)	6.92 ± 0.19 (6.79 - 7.13)	2.04 ± 0.20 (1.89 - 2.27)
Adult Female (Body)	7.06 ± 0.11 (6.99 - 7.19)	2.34 ± 0.29 (2.01 - 2.56)	7.18 ± 0.26 (6.89 - 7.40)	2.73 ± 0.08 (2.65 - 2.81)
Adult Male (Wing expansion)	-	15.88 ± 1.39 (14.92 - 17.48)	-	15.97 ± 1.69 (14.27 - 17.65)
Adult Female (Wing expansion)	-	17.36 ± 0.44 (16.98 - 17.84)	-	17.40 ± 0.55 (16.94 - 18.00)

*Mean of 10 individuals (n=10) Values in the parenthesis = Range

Table 4 : Weight (mg) of different life stages of pink bollworm on seed bits (natural food) of different *Bt* and non-*Bt* cotton of Jadoo

Weight (mg)				
Life stages	**Jadoo BG II**		**Jadoo non-*Bt***	
	Mean ± S.D	**Range**	**Mean ± S.D**	**Range**
Larva*				
III instar	12.90 ± 0.28	12.58 - 13.12	14.59 ± 0.64	13.87 - 15.09
IV instar	29.39 ± 1.89	27.29 - 30.95	32.80 ± 2.59	29.87 - 34.80
Pupa*				
Male	17.56 ± 1.15	16.27 - 18.49	19.37 ± 1.53	17.89 - 20.95
Female	19.82 ± 0.95	18.83 - 20.72	22.64 ± 1.12	21.73 - 23.89
Adult*				
Male	15.20 ± 0.91	14.27 - 16.09	18.26 ± 0.94	17.21 - 19.01
Female	16.45 ± 1.25	15.49 - 17.87	18.34 ± 1.17	16.99 - 19.04

*Mean of 10 individuals (n=10)

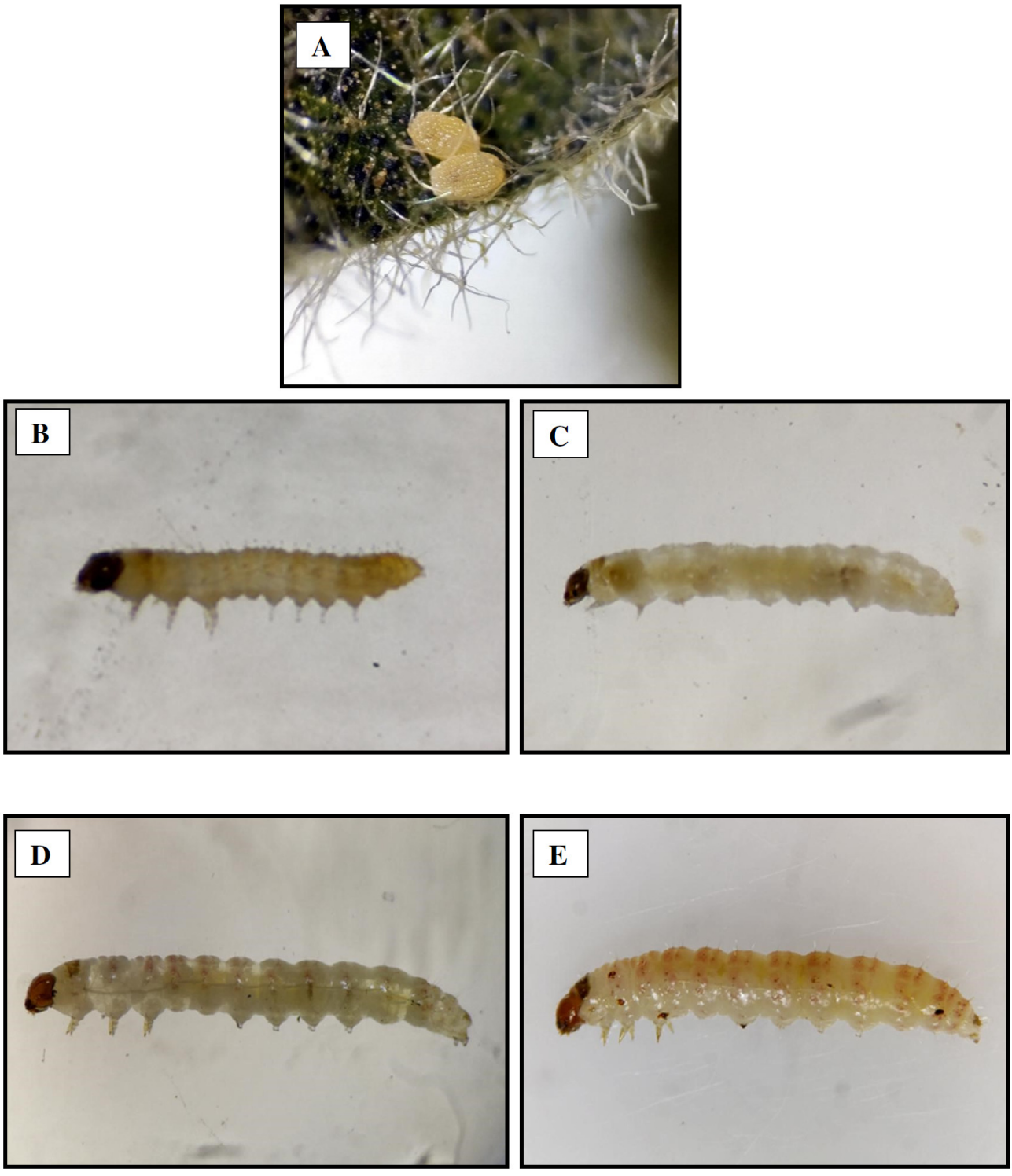

Figure 1a: Life stage of PBW (Microscope zoom magnification: 0.78, microscope main objective magnification: 0.63 and Camera exposure: 100.0 ms). A) Egg B) First instar C) Second instar D) Third instar and E) Fourth instar

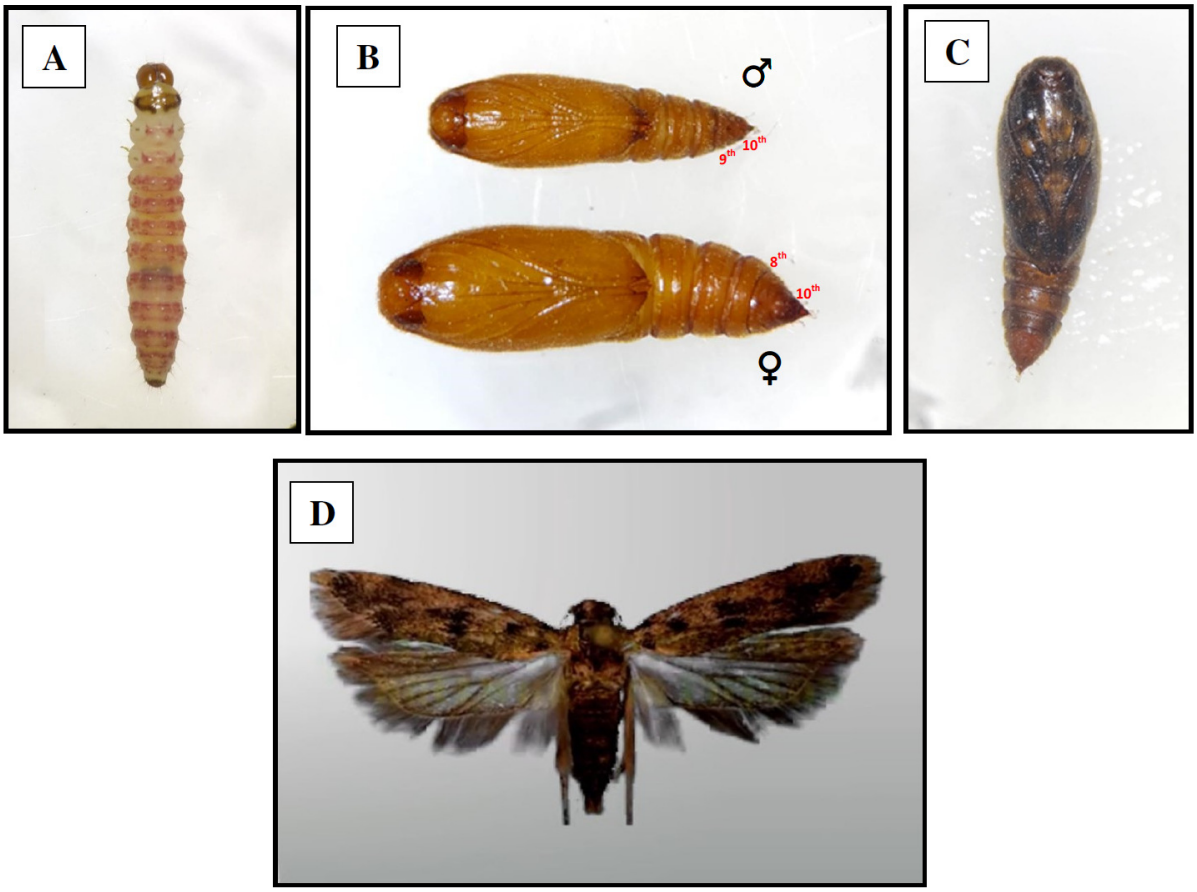

Figure 1b: Life stages of PBW (Microscope zoom magnification: 0.78, microscope main objective magnification: 0.63 and Camera exposure: 100.0 ms). A) Prepupa B) Male and Female pupa Matured pupa C) Matured pupa and D) Adult moth

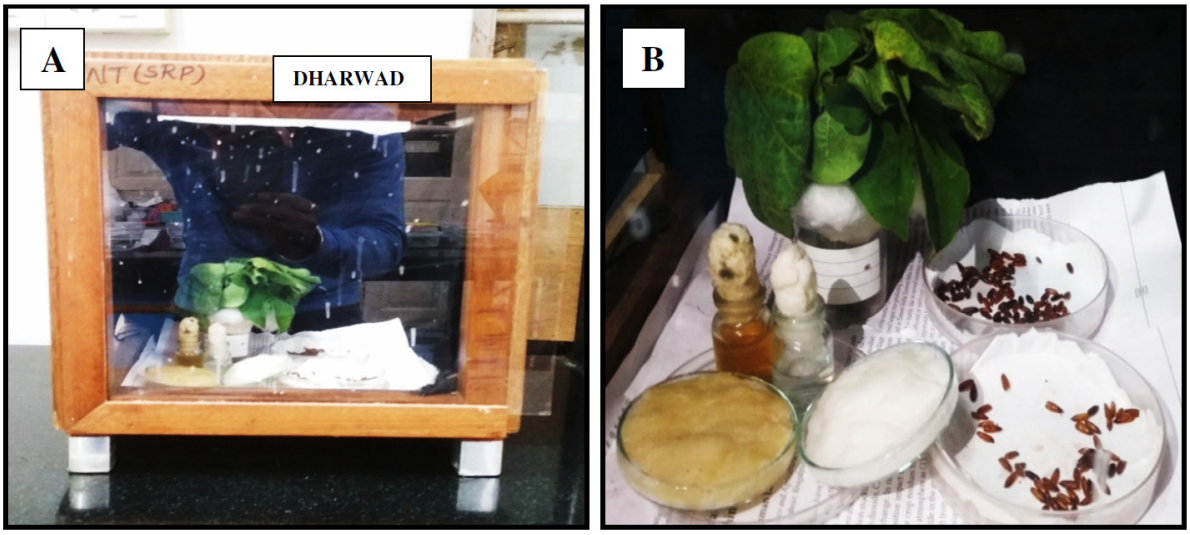

Figure 2: A) and B) Adult rearing and oviposition setup

List of Symbols and Abbrevations

%	:	Per cent
±	:	Plus or minus
°C	:	Degree Celsius
ARS	:	Agricultural Research Station
Bt	:	*Bacillus thuringiensis*
PBW	:	Pink bollworm
et al.,	:	Co-workers

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Authors' contributions

Shashank D. U. conducted the experiments and gathered data, while Poornima V. Matti formulated and designed the experiments. S.B. Patil and Manjula S. Maralappanavar provided reagents, materials, and analysis tools, and also participated in revising the paper. All authors reviewed and consented to the final manuscript.

Competing interests

The authors declare that they have no competing interests

References

Agarwal, R.A., Gupta, G.P. and Grag, D.O. (1984). Cotton pest management in India. *Research Publication*, Azad Nagar, Delhi, India, pp. 1-191.

Badiger, H.K. (2010). Comparative efficacy of interspecific cotton hybrids containing single and stacked *Bt* genes against pink bollworm *Pectinophora gossypiella* (Saunders) and Tobacco caterpillar, *Spodoptera litura* (Fab.), *M. Sc. (Agri) Thesis*, University of Agricultural Sciences, Dharwad, Karnataka, India.

Dharajothi, B., Naik, V.C., Kranthi, S., Kranthi, K.R., Valarmathi, R. (2016). Viable mass production method for cotton pink bollworm, *Pectinophora gossypiella* (Saunders). *The Journal of Basic and Applied Zoology*, 1(73): 9-12.

Dharajothi, B., Valarmathi, R., Nagarajanm, T. and Rajan, T. (2010). Larval sex differentiation of pink bollworm–an easy tool for pairing of adults for mass rearing. Centre Institute for Cotton Research, Newsletter, *Nagpur*, 26(3): 1-5.

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

Fand, B.B., Nagrare, V.S., Gawande, S.P., Nagrale, D.T., Naikwadi, B.V., Deshmukh, V., Gokte-Narkhedkar, N. and Waghmare, V.N. (2019). Widespread infestation of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechidae) on *Bt* cotton in Central India: a new threat and concerns for cotton production. *Phytoparasitica*, 47(3): 313-325.

Ghosh, S.K. (2001). GM crops: Rationally irresistible. *Current Science*, 81(6): 655-660.

Mohan, K.S., Ravi, K.C., Suresh, P.J., Sumerford, D. and Head, G.P. (2016). Field resistance to the Bacillus thuringiensis protein *Cry*1Ac expressed in Bollgard® hybrid cotton in pink bollworm, *Pectinophora gossypiella* (Saunders), populations in India. *Pest Management Science*, 72(4): 738-746.

Nagamandla, R.S. and Maheswari, T.U. (2021). Bionomics of pink bollworm, *Pectinophora Gossypiella* (Saunders) on cotton and its alternate hosts. *Indian Journal of Entomology*, 83(4): 456-550.

Naik, V.C., Kumbhare, S., Kranthi, S., Satija, U. and Kranthi, K.R. (2018). Field evolved resistance of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), to transgenic *Bacillus thuringiensis* (*Bt*) cotton expressing crystal 1Ac (*Cry*1Ac) and *Cry*2Ab in India. *Pest Management Science*, 74(11): 2544-2554.

Navya, N. (2019). Studies of pink bollworm (*Pectinophora gossypiella* Saunders.) in *Bt* and non-*Bt* cottons. *M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Dharwad, Karnataka, India.

Pradhan, K. (2019). Studies on diapause behaviour of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *M. Sc (Agri.) Thesis*, University of Agricultural Sciences, Raichur, Karnataka, India.

Rajput, I.A., Lodhi, A.M., Syed, T.S., Abro, G.H. and Khatri, I. (2019). Comparative Biology of Pink Bollworm, *Pectinophora gossypiella* Saund. on *Bt*. and Non-*Bt*. Cotton: Pink Bollworm Biology on Cotton. *Biological Sciences-Pakistan Journal of Scientific and Industrial Research*, 62(2): 116-121.

Rakhesh, S. (2021). Population dynamics and management of pink bollworm, *Pectinophora gossypiella* (Saunders) in *Bt* cotton. *M. Sc. (Agri.) Thesis*, University of Agricultural sciences, Raichur, Karnataka, India.

Sapna, P. (2014). Bioecology of cotton pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *M. Sc. (Agri.) Thesis*, University of Agricultural sciences, Raichur, Karnataka, India.

Shah, M.A., Memon, N., Shaikh, A.M. and Mal, B. (2013). Biology of Pink bollworm (*Pectinophora gossypiella*) Lepidoptera: Gelechiidae on different temperatures under controlled conditions. *Sindh University Research Journal,* 45(2): 321-324.

Shrilakshmi, R.G. (2021). Studies on *Cry* toxin resistance and climatic change impact with reference to pink bollworm *Pectinophora gossypiella* (Saunders) on *Bt* cotton. *Ph. D Thesis*, University of Agricultural Sciences, Dharwad, India.

Shrinivas, A.G., Hanchinal, S., Hurali, S. and Beldhadi, R. (2019). Comparative biology of pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) on different hosts. *Journal of Entomology and Zoology Studies*, 7(1): 1053-1060.

Tanani, M.A. and Bakr, N.A. (2018). Effectiveness of the chitin synthesis inhibitor, diofenolan, on survival and development of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera:

Gelechiidae). *Journal of Entomology and Zoology Studies*, 6(4): 1209-1219.

Vijaykumar, D.K. (2019). Biology and life-fecundity of pink bollworm, *Pectinophora gossypiella* (Saunders) on cotton at different temperature levels, *M. Sc. (Agri.) Thesis*, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India.

Zinzuvadiya, H.D., Desai, H.R., Lakum, M.B. and Rajkumar, B.K. (2017). Biology of pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) on artificial diet under controlled condition. *Trends in Biosciences*, 10(25): 5363-5371.