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Original article

Using two-sex life table tools to compare the population parameters of *Oxycarenus hyalinipennis* costa (Lygaeidae: Hemiptera) when fed on *Bt* and non-*Bt* cotton seeds



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ABSTRACT

Cotton crop (*Gossypium hirsutum* L.) is considered economy crop worldwide especially in Pakistan. Cotton plays strong role in proportion of GDP and foreign exchange earnings. In Pakistan, around 55% cotton is exporting fiber commodity and 15 % area of cultivation is devoted to cotton crop especially *Bt* cotton. However, *Bt* cotton suppress the chewing pests but give a favorable niche to sucking pests. Among sucking pests, the *Oxycarenus hyalinipennis* Costa is emerging sucking pest in *Bt* cotton, and damaging the more than 40 different plants. Study regarding preference of seed and two-sex life table traits of *O. hyalinipennis* is necessary for its eco-friendly management. Therefore, this explain the preference of cotton seeds e.g., CIM-620 (non-*Bt* variety), CIM-717 (non-*Bt* variety), *Bt*-CIM-632 and *Bt*-Cyto-179 by *O. hyalinipennis*, as well as its fitness on these seeds under laboratory condition. Results indicated that both males and females preferred *Bt*-Cyto-179 for ovipositing as compared to other three varieties. The developmental time from eggs to males (176.85 ± 7.43) and females (184.94 ± 7.85) were significantly longer on other varieties as compared to *Bt*-Cyto-179. The *O. hyalinipennis* females produce significantly more eggs on *Bt*-Cyto-179 (215 ± 2.34 eggs female⁻¹) than those on other three seeds e.g., CIM-620, CIM 717, and *Bt*-CIM-632. In conclusion, *Bt*-Cyto-179 was the more suitable varieties to attract and rear *O. hyalinipennis* than CIM 632, and CIM 717 seeds. And our study suggests that *Bt*-Cyto-179 can be used as a bait crop to control *O. hyalinipennis* and rearing purpose for molecular studies.

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1. Introduction

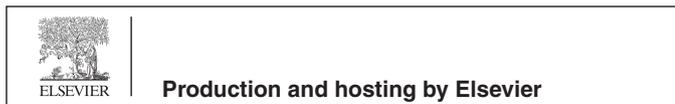
Cotton, *Gossypium hirsutum* L., especially *Bt*-cotton is considered economical crop and provide major income source to people all

over the world especially in Pakistan (Ahmad et al., 2020; Arshad et al., 2021). Last few years, the area of *Bt*-cotton cultivation decreased in Pakistan because of various factors that affecting the cotton productivity in Pakistan (Arshad et al., 2021; Khan et al., 2014a), as one of insect pest because cotton crop is more susceptible nature. However, previous literatures proved that *Bt* cotton suppressed bollworms but found less effective against sucking pests except a few thrips species (Jaleel et al., 2014; Razaq et al., 2019). Another reason is unselective and blind application of insecticides affecting the environmental safety (Poudel et al., 2020); moreover, it also caused resistance in insects that reduced their vulnerability to the insecticides (Jaleel et al.,

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2020; Lei et al., 2021; Saeed et al., 2016). However, nowadays in Pakistan, among sucking pest of cotton, the *Oxycarenus hyalinipennis* Kirby (Lygaeidae: Hemiptera) is considered damaging bug in cotton crop.

Now, the *O. hyalinipennis* has gained the status of major pests of cotton crop in Pakistan, as well as in other countries e.g., China, Turks, Islands of U.S. Bahamas, Caicos, and Cayman (Colmenárez et al., 2020; Stocks and Peña, 2013). Although it is a well-known pest of crops of family Malvaceae, its infestation has been observed on other crops like guava, moringa, mango, okra, chilies and lemon (Naeem-Ullah et al., 2020). It's adults as well as nymphs are responsible for affecting the quality and quantity of yield and oil extraction of cotton seeds. Its adults and nymphs sociably feeds on squares of cotton plant, resulting in pale yellow color of squares and then shedding of infested squares (Rajendran et al., 2018). Lot of factors may affect the population parameters of dusky cotton bug e.g. adaptation of novel techniques in cotton crop like early sowing of *Bt*-cotton and extreme use of insecticides (Khan and Ghramh, 2021a; Rocha-Munive et al., 2018). Such factors are creating niche for a survival of dusky cotton bug and this insect pest is gaining the status of economically important pests in Pakistan (Khan and Ghramh, 2021b; Naeem-Ullah et al., 2020).

Use of insecticides is considered a reliable method against this insect pest in Pakistan and other developing countries (Ghramh et al., 2020; Hashmi and Khan, 2011). But excessive use of insecticides causes serious environmental and health problems for human being and non-target organisms. Frequent use of insecticides also causes the problems of resistance development in insects (Ghramh et al., 2019; Khan et al., 2014b). Dusky cotton bug has also developed resistance against different group of insecticides (Banazeer et al., 2021). However, the alternative control strategy is needed for the management of dusky cotton bug.

Basic information of insect pests especially *O. hyalinipennis* are important in the integrated management program (Ijaz and Shad, 2021a; Jaleel et al., 2018; Sarmad et al., 2020). However, the fitness of bugs can be better explained by using two-sex life table tools to find the susceptible point (Huang and Chi, 2012). The study regarding life table parameters of *O. hyalinipennis* have been explained on different hosts including cotton (Ananthakrishnan et al., 1982; Banazeer et al., 2021; Ijaz and Shad, 2021b; Wazir and Shad, 2021); however, few studies are available on cotton seeds preference by *O. hyalinipennis* and its fitness. Considering the importance of cotton crop this research explains the behaviour of *O. hyalinipennis* on two non-*Bt* (CIM 620 and CIM 717) and *Bt* (*Bt*-CIM-632 and *Bt*-Cyto-179) varieties in the laboratory.

2. Materials and methods

2.1. Insect collection, rearing, and seeds

The *O. hyalinipennis* was collected from different cotton fields and maintained in laboratory of Entomology, Central Cotton Research Institute (CCRI), Multan, Punjab, Pakistan. Dusky cotton bugs were shifted into jugs having dimension 14 × 24 cm. Temperature (26 ± 2 °C) and humidity (60 ± 5%) were maintained in laboratory using air conditioner (Haier Pear 18HFAA, Ind. Ltd Pk) and humidifier (Life Care 600 Ind. Ltd Pk) with photoperiod 15: 9 h L: D. The *O. hyalinipennis* were fed on soaked seed of each variety. Population of dusky cotton bugs were reared on each seed e.g., two non-*Bt* (CIM 620 and CIM 717) and *Bt* (*Bt*-CIM-632 and *Bt*-Cyto-179) for two generation to maintain homogeneous population and make them suitable for laboratory study.

2.2. Data recording

After populations of *O. hyalinipennis* acclimatized, the two sex life table parameters were studied on two non-*Bt* (CIM 620 and CIM 717) and *Bt* (*Bt*-CIM-632 and *Bt*-Cyto-179) seeds. Hundred eggs of dusky cotton bugs on each seed were collected and shifted on respective seeds in 14 × 24 cm plastic jars to record the two-sex life table parameters. After adult formation, A pairs (male: female) of adults shifted into a Petri dish (9 × 1.5 cm) for fecundity. Total 10 replication was done on each cotton seeds.

2.3. Protein analysis

Buffers were used to maintain enzyme nature during crushing of tested seeds. The ingredients for extracting protein were used as distilled water, Na₂HPO₄12H₂O, NaH₂PO₄2H₂O, blue dye, phosphoric acid, and ethanol. As first 8.10 g of Na₂HPO₄2H₂O crystals was poured into volumetric flask having water 250 ml and we designated as "A". And 0.35 g of Na₂HPO₄2H₂O was dissolved in 200 ml of water and we designated as "B". Then, 20 ml solution of "A" and 80 ml solution of "B" were mixed for maintained pH 7. This solution was used to find out the protein values in seed contents.

2.4. Statistical analysis

The statistics software was used to calculate the mean and the LSD test variation among seeds treatments. The two-sex life table traits (Chi, 1988; Chi and Liu, 1985) were calculated using TWO-SEX LIFE TABLE program (Chi, 1988; Liu et al., 2017). The values of population and age stage parameters R_0 , and S_{xj} , f_{xj} , l_x , m_x , e_{xj} , v_{xj} respectively were calculated as described in the methodology of (Chi, 1988; Jaleel et al., 2018). The following equations explained the two-sex life table calculations.

$$l_x = \sum_{j=1}^k S_{xj} \tag{1}$$

$$m_x = \sum_{j=1}^k S_{xj} f_{xj} / \sum_{j=1}^k S_{xj} \tag{2}$$

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \tag{3}$$

$$\sum_{x=0}^{\infty} e^{r(x+1)} l_x m_x = 1 \tag{4}$$

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^k s'_{iy} \tag{5}$$

where s'_{iy} of *O. hyalinipennis* is prospect of x and j that will remain to i and stage y as described in the study of Tuan et al. (2014),

$$v_{xj} = \frac{e^{r(x+1)}}{S_{xj}} \sum_{i=x}^{\infty} e^{-r(x+1)} \sum_{y=j}^k s'_{iy} f_{iy} \tag{6}$$

3. Results

3.1. Life history parameters

Longevity of different nymphal instars on different seeds was significantly different. Larval instars' duration increases when reared on *Bt*-Cyto-179 (Table 1). First larval instar developed

Table 1
Influence of *Bt* and non-*Bt* seeds on the biological parameters of the *O. hyalinipennis*.

Parameters	Cotton Seeds			
	Non- <i>Bt</i>		<i>Bt</i>	
	CIM 620	CIM 717	<i>Bt</i> -Cyto-179	CIM 632
Egg (d)	5.06 ± 0.19b (n: 100)	4.62 ± 0.23c (n:100)	6.37 ± 0.18a (n:100)	5.09 ± 0.23b (n:100)
1st instar (d)	4.16 ± 0.18c (n: 74)	5.39 ± 0.29b (n:66)	5.58 ± 0.30a (n:93)	4.05 ± 0.23d (n:66)
2nd instar (d)	4.12 ± 0.29b (n: 50)	4.38 ± 0.29a (n:47)	3.61 ± 0.29c (n:72)	4.17 ± 0.25b (n:47)
3rd instar (d)	3.21 ± 0.09d (n: 47)	3.94 ± 0.15b (n:40)	3.58 ± 0.11c (n:60)	4.08 ± 0.11a (n:40)
4th instar (d)	4.83 ± 0.17c (n: 47)	5.35 ± 0.18b (n:40)	5.55 ± 0.12a (n:60)	4.08 ± 0.17d (n:40)
5th instar (d)	5.61 ± 0.18c (n: 41)	5.59 ± 0.25c (n:40)	8.13 ± 0.37a (n:60)	7.20 ± 0.25b (n:40)
Male adult longevity (d)	51.55 ± 1.09c (n: 29)	48.54 ± 1.14d (n:19)	64.92 ± 2.02a (n:36)	62.76 ± 7.31b (n:19)
Female adult longevity (d)	85.25 ± 6.89d (n: 12)	100.29 ± 2.27c (n:21)	116.71 ± 2.34a (n:24)	109.21 ± 7.76b (n:21)
TPOP (d)	30.75 ± 0.64d (n: 12)	36.48 ± 0.64c (n:21)	39.62 ± 0.78a (n:24)	37.79 ± 0.20b (n:21)
APOP (d)	4.00 ± 0.25c (n: 12)	4.71 ± 0.53b (n:21)	4.46 ± 0.22b (n:24)	5.95 ± 0.50a (n:21)
Oviposition (d)	4.75 ± 1.18c (n: 12)	5.90 ± 0.58b (n:21)	6.88 ± 0.44a (n:24)	5.68 ± 0.22b (n:21)
Fecundity Egg/Female	126.50 ± 9.58d (n: 12)	178.64 ± 17.90b (n:21)	215.85 ± 9.27a (n:24)	162.95 ± 6.27c (n:21)

n: represent the no. of individual, **APOP**: Adult pre-oviposition period of a female adult, **APRP**: Adult pre-reproduction period of a female adult, The standard errors of the mean values of pre-adult development period and total longevity of male and females were estimated by using 200,000 bootstrap replicates. Means marked with different letters are significantly different between three treatments using the paired bootstrap test at the 5% significant level.

longer on *Bt*-Cyto-179 than other tested seeds ($P < 0.0001$). Similarly, same pattern was observed in other instars. Adults of *O. hyalinipennis* lived longer on *Bt*-Cyto-179 ($P < 0.0001$). The APOP period was significantly longest on CIM 632 ($P < 0.001$). While, maximum TPOP duration was recorded on *Bt*-Cyto-179 seeds as compared to other three seeds ($P < 0.0001$) (Table 1). Number of eggs increased dynamically as to optimum condition/maximum numbers achieved on *Bt*-Cyto-179 (Table 1). Fecundity was significantly highest when dusky cotton bugs fed on *Bt*-Cyto-179 seeds as compared to three other seeds ($P < 0.0001$) (Table 1).

3.2. Population parameters

Longer mean relative growth rate was calculated on seeds *Bt*-Cyto-179 ($P < 0.01$). Significantly highest mean intrinsic growth rate was found on *Bt*-Cyto-179 as compared to other three tested seeds ($P < 0.001$) (Table 2). The life table parameters (Table 2) showed maximum values on *Bt*-Cyto-179 as compared to other three cotton seeds ($P < 0.0001$). The double time of *O. hyalinipennis* was significantly longer on CIM 620 (Table 1). Curves of s_{xj} for *O. hyalinipennis* development on *Bt* and non-*Bt* cotton seeds were different (Fig. 1). Egg laying of *O. hyalinipennis* at x and j stages were different (Fig. 2). The L_x and M_x of *O. hyalinipennis* were greatly influenced by *Bt* and non-*Bt* cotton seeds (Fig. 2). The E_x of *O. hyalinipennis* longer on *Bt*-Cyto-179 seeds (Fig. 3). The V_{xj} of *O. hyalinipennis* adults have shown in the Fig. 4.

3.3. Protein contents

The protein contents were maximum in *Bt*-Cyto-179 ($P < 0.0001$) as compared to other three seeds (Fig. 5).

Table 2
Influence of *Bt* and non-*Bt* seeds on the population parameters of the *O. hyalinipennis*.

Parameters	Different cotton seeds			
	CIM 620	CIM 717	CIM 632	<i>Bt</i> -Cyto-179
r (per day)	0.06	0.07	0.07	0.07
λ (per day)	1.06	1.07	1.07	1.08
GRR (offspring)	123.86	154.94	89.23	155.35
R₀ (offspring/individual)	15.18	37.52	30.96	51.80
Mean Generation Time (T) (Days)	44.49	51.08	49.59	56.39
Doubling Time (D) (Days)	11.34	9.77	10.01	9.90

r; The intrinsic rate of increase (per days).
λ; The finite rate of increase (per days).
GRR; Gross reproductive rate (offspring).
R₀; The net reproductive rate (offspring/individual).
T; The mean generation time (days).

4. Discussion

Several studies explained the host influence on the development, demographic outcomes, and fitness of insects (Jaleel et al., 2018). This study was carried out by the considering the importance of host preference and the response of *O. hyalinipennis*. The difference in fitness and population parameters explains the preference of seeds. Growth rate in *O. hyalinipennis* enhanced on favorable hosts due to sufficient proteins in activation energy. As lot of studies revealed that *Bt* varieties of cotton are not resistant against sucking pests which resulted in enhanced metabolic rate, similar results were found in our study as *Bt*-Cyto-179 was more susceptible for dusky cotton bug.

Female adults kept specific behaviour of host selection for oviposition and development of newborns (Jaleel et al., 2018; Valladares et al., 2020). The olfaction and gustation sense of insect pests play an important role in their host selection and fitness of immature (Anderson and Anton, 2014; Jaleel et al., 2021a; Jaleel et al., 2021b). Maximum number of eggs was achieved on favorable seeds e.g., *Bt*-Cyto-179, this is possibly due to available seed contents. The adult fitness and fecundity are two main parameters for the increasing population of insects (Huang and Chi, 2012; Sarmad et al., 2020). A number of sucking pests undergo severe stress on unsuitable hosts.

The population parameters e.g., “r” can be better explained using two-sex programs (Huang and Chi, 2012; Jaleel et al., 2018). Fitness of insect pest explained the role of r, if its value becomes maximum than 0, than food of insect consider favorite or suitable for development and survival of insect (Chi et al., 2020). Another factor of increasing population is “R₀” that explained the role of diet or food in high fecundity. In our

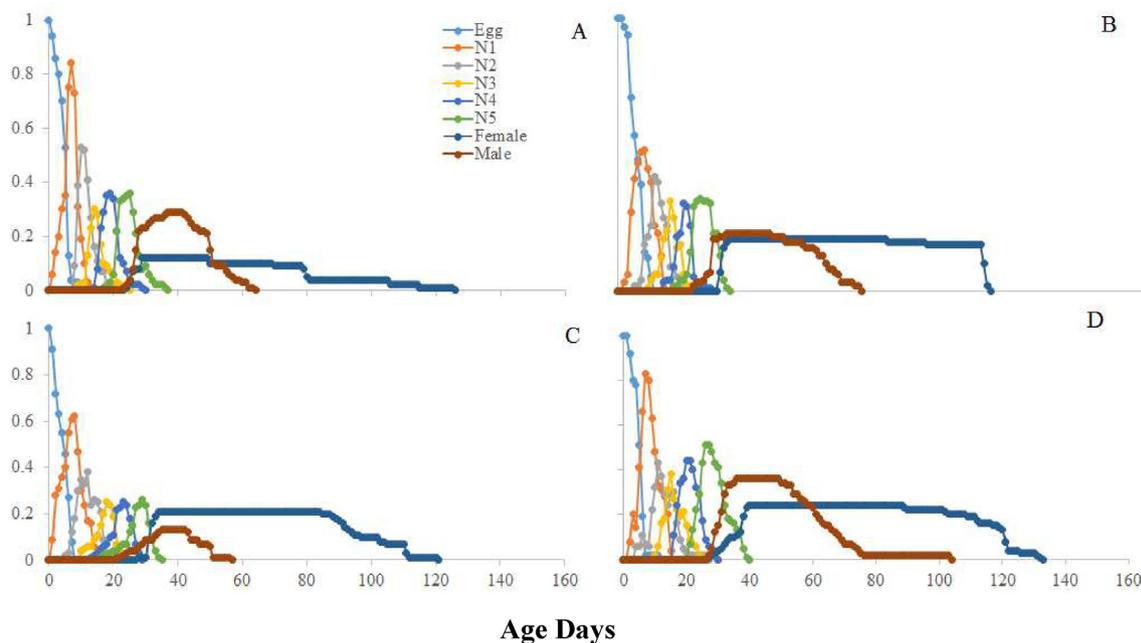


Fig. 1. Influence of *Bt* and non-*Bt* seeds on the age-stage-specific survival rate (s_{xj}) of the *O. hyalinipennis*. A: CIM 620, B: CIM 632, C: CIM 717, and D: *Bt*-Cyto-179, N1: 1st Instar Nymph, N2: 2nd Instar Nymph, N3: 3rd Instar Nymph, N4: 4th Instar Nymph, N5: 5th Instar Nymph.

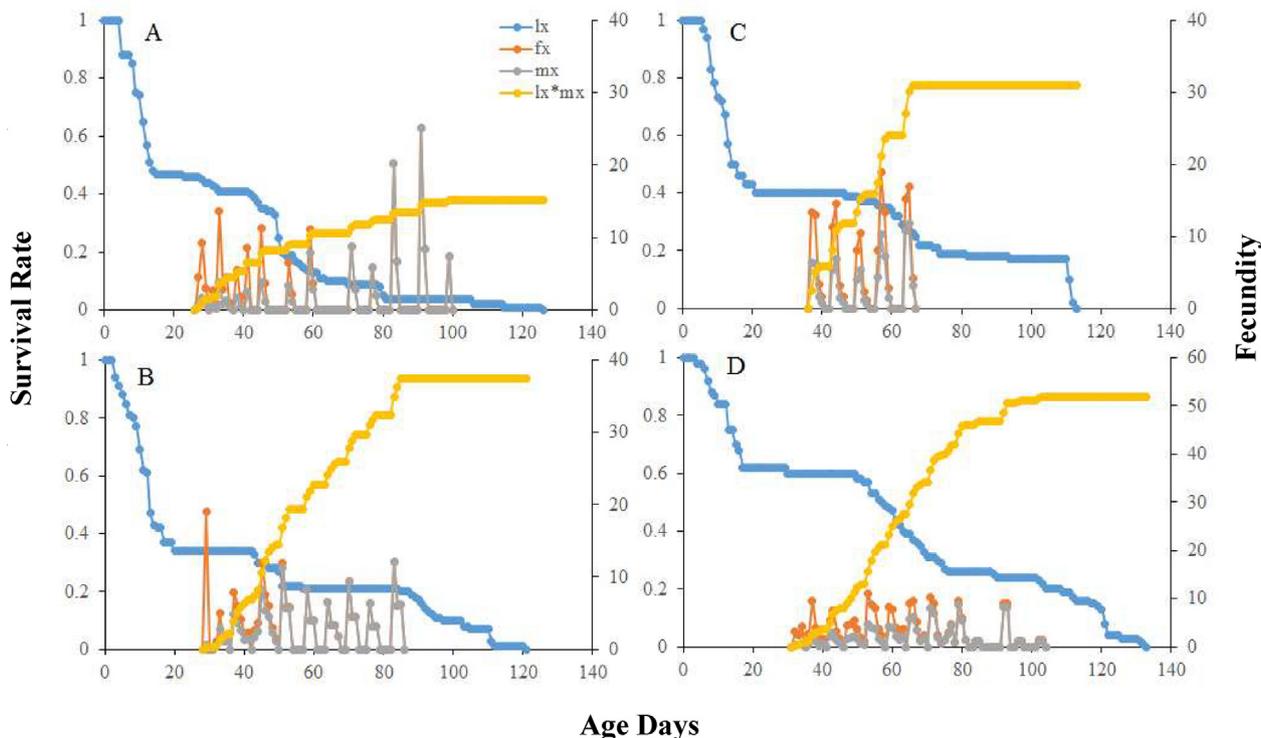


Fig. 2. Influence of *Bt* and non-*Bt* seeds on the Age-specific survival rate (l_x), female age-specific fecundity (f_x), age-specific fecundity (m_x), and age-specific maternity ($l_x \cdot m_x$) of the *O. hyalinipennis* A: CIM 620, B: CIM 632, C: CIM 717, and D: *Bt*-Cyto-179.

study, the maximum eggs of *O. hyalinipennis* were recorded on *Bt*-Cyto-179 (Table 1). The results of this experiment summarized those seeds of *Bt*-Cyto-179 was found better for development of *O. hyalinipennis* so its life cycle becomes shorter with

increase of fecundity. These results will be helpful in the management of *O. hyalinipennis* in Punjab, Pakistan. so further work would be carried out to find the molecular studies of *O. hyalinipennis*.

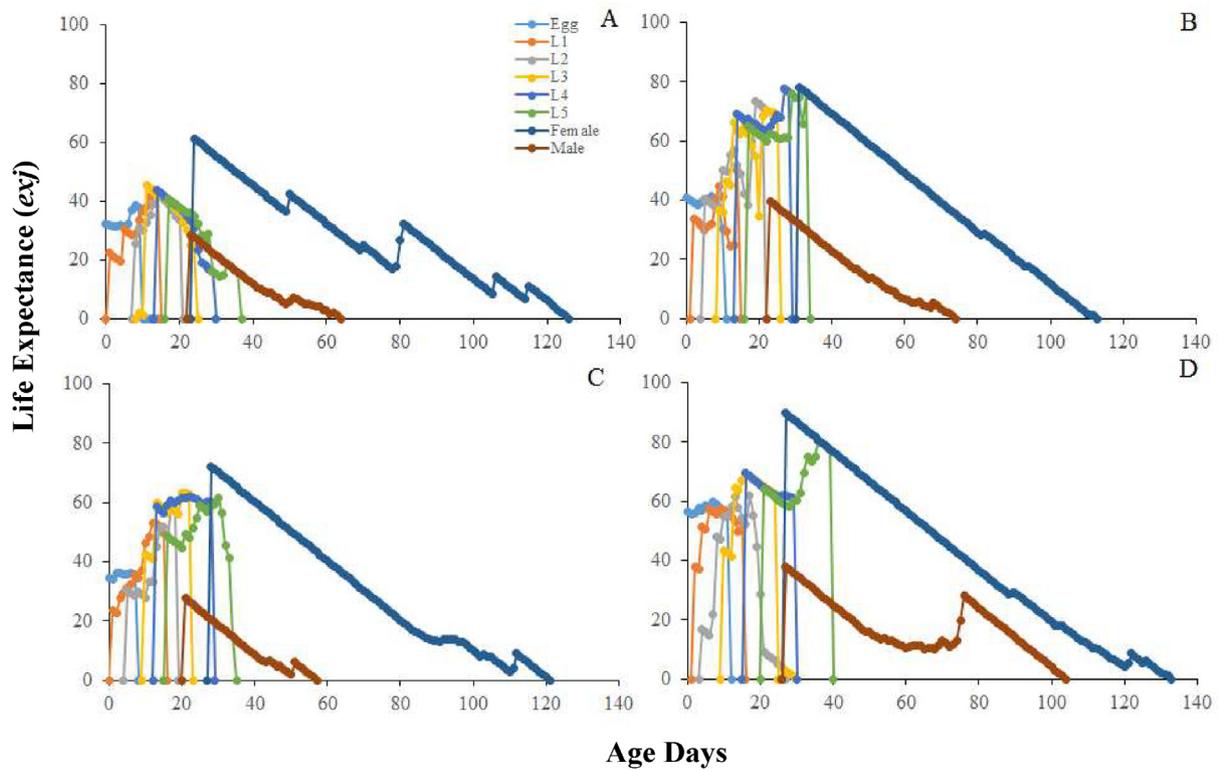


Fig. 3. Influence of *Bt* and non-*Bt* seeds on the age-stage-specific life expectancy (e_{xj}) of the *O. hyalinipennis* A: CIM 620, B: CIM 632, C: CIM 717, and D: *Bt*-Cyto-179, NI: 1st Instar Nymph, N2: 2nd Instar Nymph, N3: 3rd Instar Nymph, N4: 4th Instar Nymph, N5: 5th Instar Nymph.

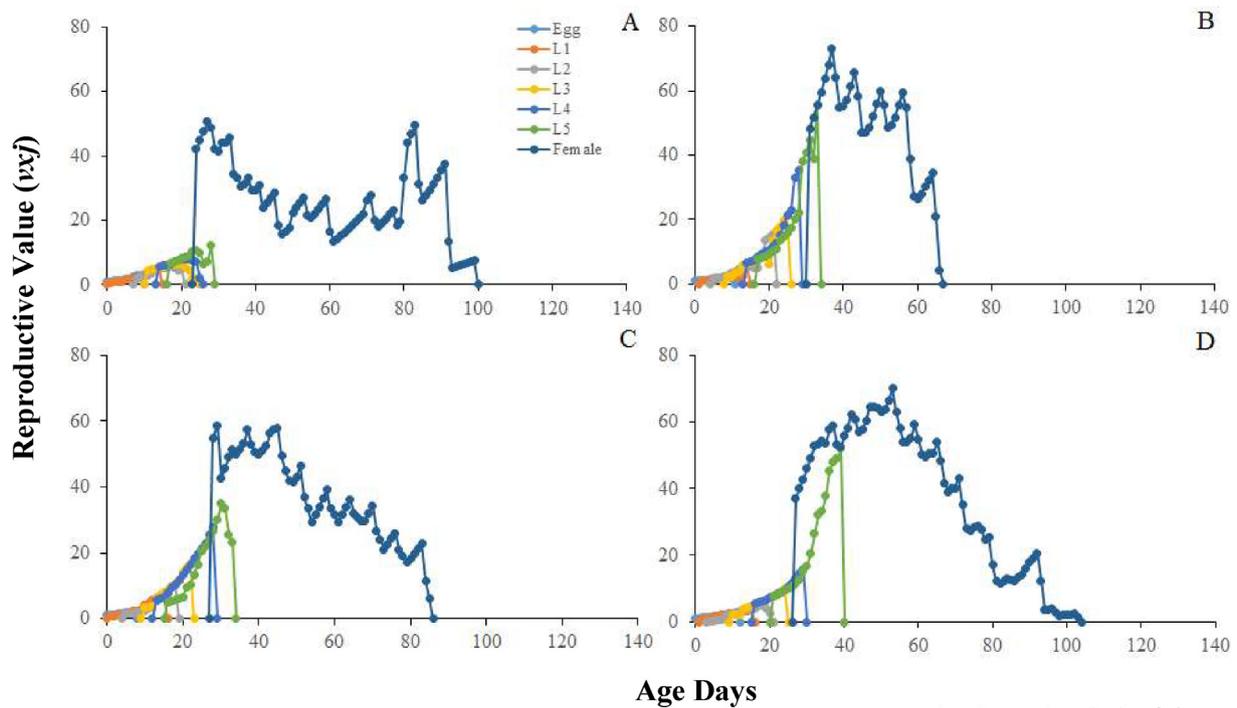


Fig. 4. Influence of *Bt* and non-*Bt* different temperatures on the age-stage reproductive value (v_{xj}) of the *O. hyalinipennis*. A: CIM 620, B: CIM 632, C: CIM 717, and D: *Bt*-Cyto-179, NI: 1st Instar Nymph, N2: 2nd Instar Nymph, N3: 3rd Instar Nymph, N4: 4th Instar Nymph, N5: 5th Instar Nymph.

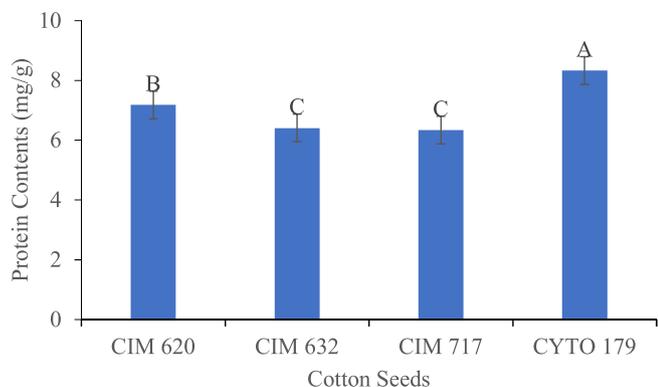


Fig. 5. Protein Contents in *Bt* and non-*Bt* of cotton. Different letter bar represents the different values in the bars.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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