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Original article

Effects of seasonal variation on the biology and morphology of the dusky cotton bug, *Oxycarenus laetus* (Kirby)



Muhammad Mithal Rind^a, Samy Sayed^b, Hakim Ali Sahito^{a,c}, Khalid Hussain Rind^{d,*}, Nadir Ali Rind^d, Akhtar Hussain Shar^d, Hidayat Ullah^d, Peter Ondrisik^e, Jana Ivanic Porhajsova^e, Zihu GUO^f, Mohamed Shahren^g

^a Department of Zoology, Faculty of Natural Sciences, Shah Abdul Latif University, Khairpur, Sindh, Pakistan

^b Department of Science and Technology, University College-Ranyah, Taif University, B.O. Box 11099, Taif 21944, Saudi Arabia

^c Laboratory of Entomology, Date Palm Research Institute, Shah Abdul Latif University, Khairpur, Sindh, Pakistan

^d Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad, Pakistan

^e Department of Environment and Biology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra, Slovakia

^f College of Life Science, Northwest A & F University, Yangling, Shaanxi, China

^g Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt

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ABSTRACT

The dusky cotton bug (*Oxycarenus laetus*, KIRBY) a pest of several crops. The effects of winter and summer on the biology and morphology was investigated. The sampled eggs of dusky cotton bug (DCB) were kept under controlled environment for biological and morphological investigations. In winter, the mating duration of DCB was observed longer significantly (74.2 days), egg development period (3.93 days), an egg laying period (3.6 days) and hatching period (6.66 days) noted longer in winter season. Interestingly, average number of hatched egg (16.8 days) observed significantly higher in summer and the percentage of the hatching of eggs (81.95%) were also observed higher in summer as compare to winter. Whereas, the longevity of all nymph stages in winter longer days as compare to summer nymph stages. Moreover, differences were also observed between male and female development days between winter and summer. In the winter, female DCB development was suggestively higher as compare to summer (24 days). Whereas, the developmental days were noted considerably more in winter for males as compare to summer (14.93 days). On other hand, for morphological parameters, no differences were observed between winter and summer population of DCB.

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1. Introduction

The dusky cotton bug *Oxycarenus laetus* KIRBY (Hemiptera: Lygaeidae) is insect pest of several malvaceous crop pest (Schaefer and Panizzi, 2000; Thangavelu, 1978). The dusky cotton bug (DCB) damage the reproductive parts of the plants by sucking the cell sap, resultantly, deteriorate the quality of lint of cotton

fiber (Awan, 2013; Vennila et al., 2007a). The DCB has been reported from as a pest from different countries such as Egypt, Sudan, Uganda, Congo, Kenya, Tanzania, Angola and Malawi (Schmutterer, 1969). The *Oxycarenus* is a large genus with fifty five species, among them, at least six species has been reported as a cotton pest (Tembe et al., 2009). The DCB severely damage and spoil the lint quality of cotton crop (Henry, 1983). The DCB pest continuously damage the quality of boll, as a result, ultimately affects the weight of the cotton seed and oil content (Henry, 1983; Schaefer and Panizzi, 2000).

In Pakistan, the qualitative and quantitative losses has been reported, for instances, premature dropping of squares, flowers and minor bolls (Patil et al., 2006). Interestingly, not only adult, but the nymph stages of the DCB potentially harmful to the cotton crops which is responsible to decrease the cotton production, seed weight and oil content (6.8%; 32% and 6%) (Rajashekhargouda et al., 1984; Sewify and Semeada, 1993).

* Corresponding author at: Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad, Pakistan.

E-mail address: khalid.rind@sbbusba.edu.pk (K. Hussain Rind).

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The color of the adult DCB pest is brown to black color, but coloration of the adult pest population depends on the geographical distribution. For instances, in the Caribbean, the DCB pest is brown and African populations are black with translucent white wings (Molet et al., 2011). The abdomen of male was rounded while female truncate, whereas, the second antennal segment is yellowish in color near the base. Female adult DCB lays egg about 15 to 26 in number (Halbert and Dobbs, 2010). Adults are brownish black in color with translucent white wings and the male abdomen is rounded and female truncate (Stibick et al., 2008).

Cotton, *Gossypium hirsutum* (L) is the backbone of Pakistan's economy and known as silver fiber or "white gold" (Sahito et al., 2011; Shah et al., 2015; Tayyib et al., 2005) Pakistan is the fourth largest producer and the third main buyer of cotton all over the world (Sahito et al., 2011). The cotton crop is grown in over 60 countries with almost 32.4 million hectares, which represents about 2.5% of all cultivated land (Matthews, 1989). The textile industries are contributing largely income generation of countries as well as in achieving millennium development goals. The fiber of the cotton is also known as king of the natural fibers and contributing 68% of the overseas trade business of tropical countries including Pakistan (Khan and Khan, 1995; Pakistan, 1980). In Pakistan, the cotton crop adds 7% to the national saving from agricultural resources and about 1.7% of the GDP (Leao et al., 2018).

Therefore, the aim of this research is to study the biology and morphology of DCB under laboratory condition during the winter season for its better pest management. The Bt-cotton variety

extensively cultivated in Pakistan as a cash crop. Whereas potential effects of DCB on Bt-cotton, to date, unknown or little is known in Pakistan. Limited amount of data is present, but still much efforts are needed to evaluate the damage to the crops by this pest in early cultivated Bt-cotton variety in cotton belt zones.

2. Material and methods

2.1. Study area

The Dusky cotton bug, a cotton pest was selected to study the biology and morphology under laboratory conditions. The last nymph stages of DCB were collected from cotton crop cultivated at the experimental of Cotton Agriculture Research Station, Kotdiji, Khairpur during, 2018 (Fig. 1). Biology was studied under laboratory conditions at department of Zoology, Shah Abdul Latif University Khairpur. The pests were collected from opened or half opened bolls of cotton crop.

2.2. Biology of dusky cotton bug, *Oxycarenus laetus* (Kirby) under laboratory conditions

When adults emerged from last nymph stage and reared on the seed of cotton. When adults matured, the pair of male and female adult released for mating. Both male and female transferred to plastic jars separately reared in a plastic jars and newly opened cotton bolls were provided as food. The inner surface of the plastic

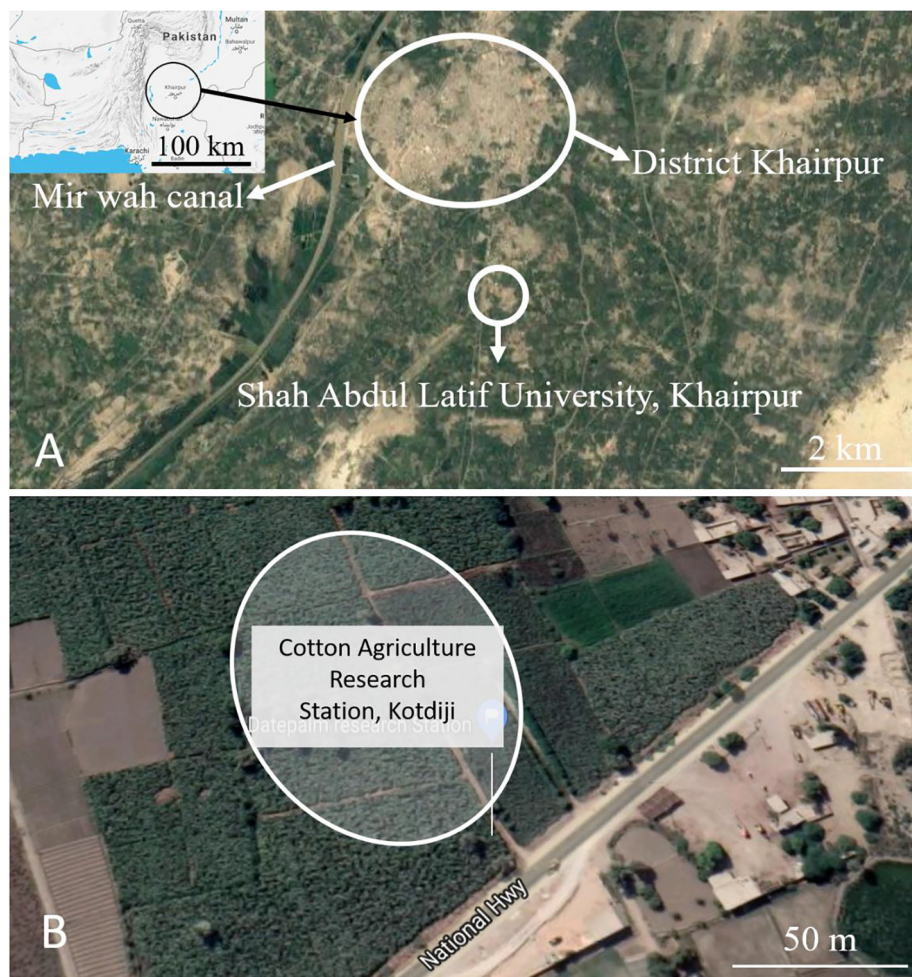


Fig. 1. Research study area of dusky cotton bug, (A) Shah Abdul Latif University Khairpur and district Khairpur, (B), sampling area, cotton agriculture research station, Kotdiji.

each jars, a blotting papers were spread out. The plastic jars were covered with muslin cloth and fastened with rubber band. The laid eggs were transferred to petri dishes with soft camel hairbrush. While, on the bottom of each petri dish, a moistened Watsman® filter paper was placed to manage an adequate humidity. After hatching nymphs were kept to medium size plastic jars for further observation. The room temperature and relative humidity were recorded with hygrometer, whereas, the photoperiod 12L: 12D (L: light and D: dark) maintained at 25 °C winter, 27 °C summer. The data was noted every day to check their changing behavior. For biological studies, from egg to adult stages were observed with compound microscope and photos were taken with same resolution and magnifications, whereas, some photos were cropped in order to see the pictures clearly.

2.3. Morphology of dusky cotton bug

Morphological characters such as color and shape of newly laid eggs were observed. The major characteristics were also observed when eggs were near to maturity. Morphological observations of newly hatched first nymph up to fifth nymph changing occurred were also observed.

2.4. Statistical analysis

The normality of the data has been checked with different parametric tests (D'Agostino-Pearson or Shapiro-Wilk normality test) and for non-parametric tests (Kruskal-Wallis test) also checked. One-way ANOVA was used to check the significance difference among all groups, students *t*-test used to compare the means of parameters. Finally, the data was statistically analyzed to compare the means of different biological parameters with statistical software package statistics GraphPad prism6.

3. Results

3.1. Biology of dusky cotton bug, *Oxycarenus laetus* (Kirby) under laboratory conditions

It was observed that the mating duration between winter and summer of DCB suggestively different ($P = 0.0022$; Fig. 2A). The results of biological parameter show that the mean male and female average mating time was 74.27 and 47.46 min. While the period of egg development was also potentially major different between winter and summer (Fig. 2B) Whereas the total period of egg lying was also differ accordingly ($P < 0.0001$) The average developmental period of adult female DCB was recorded 3.93 and 2.5 days (Fig. 2C). Interestingly, total number of eggs hatched was higher in summer as compared to winter season (Fig. 2D; $P = 0.0459$). On the other hand, the hatching time span of eggs were observed importantly higher in winter ($P = 0.0001$; Fig. 2E). The average hatching percentage of eggs were considerably higher in summer ($P = 0.0022$; Fig. 2F).

3.2. Overall fecundity and nymph developmental stages of dusky cotton bug

After hatching, first nymph was observed active and walking fast on the seed and lint of cotton crop. The average days of first nymph stage of DCB was considerably higher in winter as compare to summer ($P = 0.0459$; Fig. 3A). Whereas, second nymph stage was also observed pointedly different, in winter development of nymph took an average more days as compare to summer ($P = 0.0009$; Fig. 3B). While, third stage nymph of DCB was suggestively different, third nymph took more developmental days in winter than summer ($P = 0.0001$; Fig. 3C). Fourth nymph of DCB, on the other hand was

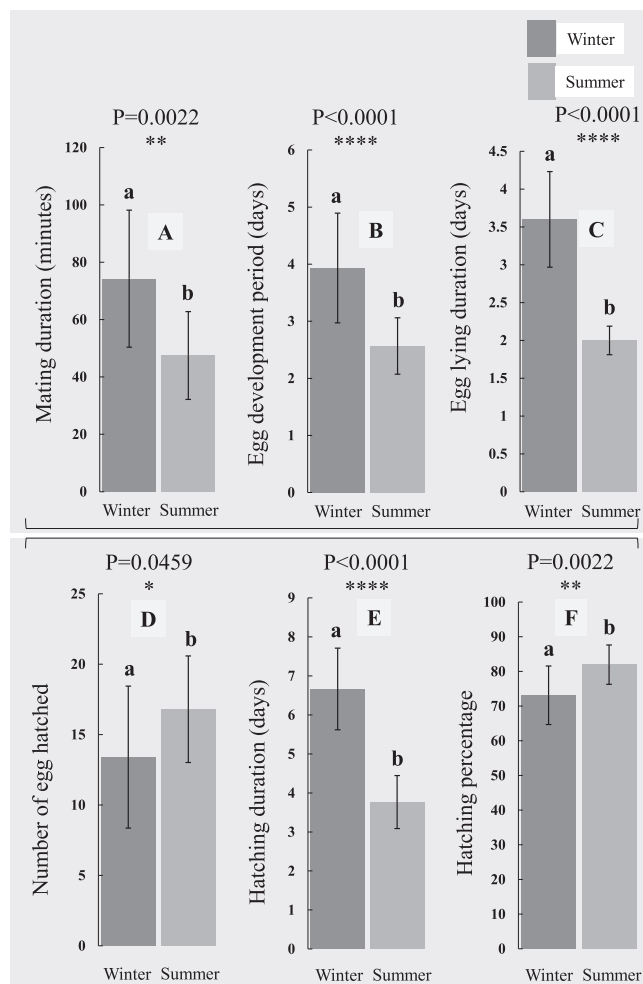


Fig. 2. Biology of DCB in winter and summer, mating duration (A), egg development period in days (B), egg lying period (C), number of egg hatched (D), hatching duration (E), and hatching percentage (F).

observed expressively different between winter and summer took more days of development ($P = 0.0003$; Fig. 3D). The fifth nymph stage considerably different between winter and summer ($P = 0.0017$; Fig. 3E). The adult developmental stages of DCB female and male were noted considerably different between winter and summer. In winter, female DCB in winter season took more days to develop as compare to development days of male DCB in winter ($P = 0.0365$; Fig. 3F). On the other hand, male DCB population was also observed different, in winter, male took more number of days to development as compare to summer ($P = 0.0211$; Fig. 3F).

3.3. Morphology characteristics of dusky cotton bug

Freshly laid eggs were observed transparent, oval, spindle shaped and tapering at each end (Fig. 4A). The eggs were then changed into light yellowish color on third day and light pink color in fifth day when they were near to hatching.

The first nymph stage was four segmented with long antennae; the last segment was thicker than other parts of antennae and rapid movement of antennae were observed (Fig. 4B). The freshly hatched nymphs were light brown then changed to head dark brown; pro-, meso-thorax dark brown and meta-thorax with abdomen light brown. The color changed up to four hours. First nymph stage was small black tail like process at the anal end of the abdomen. Second nymph stage was similar first nymph stage except size (Fig. 4C). The freshly molted third nymph stage was light

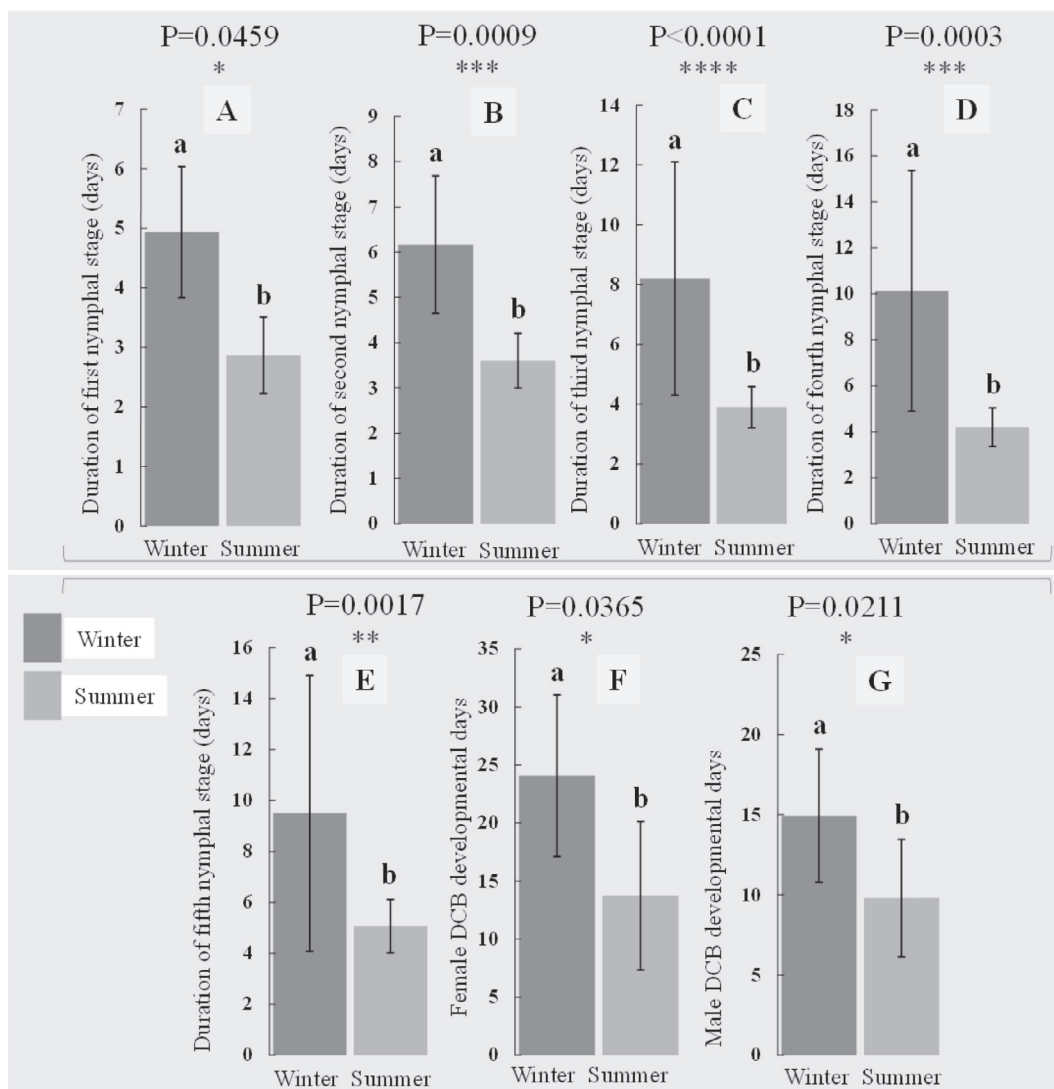


Fig. 3. Fecundity of DCB, duration of nymph stage, first (A), second (B), third (C), fourth (D), fifth (E), female DCB in winter and summer (F) and male DCB developmental days (G).

brown color, interestingly, after four days changed in to dark brown. White broad line appeared on the thorax which made different from other nymphs (Fig. 4D). Third Nymph was very active and quick movement of antennae was observed on opened bolls for search the food. Newly emerged nymphs were light brown and changed into dark brown color up to 6 h. The nymph body length was bigger than previous one with lightly developed wing pads. The leg parts such as femur wider, tibia longer, tarsus light was brown in color and fourth nymph were observed with segmented abdomen (Fig. 4E). The fifth nymph stage was light brown in color, after six hours then changed to dark brown. It has fully developed wing pads with dark brown color. The white dots were appeared on the thorax, wing pads and abdomen regions. The first and fourth segments of antennae were dark brown with second and third segment of antennae color were light brown in color (Fig. 4F).

3.4. Description of adult male dusky cotton bug

The newly emerged adult was light brown color and turned to dark brown up to 6 h. The adult male was a slender shaped abdomen and smaller size than female. It has four segmented antennae; second segment was larger and light dusky color and last one was

thicker than others. Wings were transparent and hemelytron was white dusky. The small hair was found on head and thorax. It has three pairs of legs; femur wider and dark brown; tibia and tarsus were light dusky color (Fig. 5B).

3.5. Description of adult female

The head and thorax of newly emerged female adult was light brown then changed dark brown color up to 9 h. Abdomen was brown in color. It has four segmented antennae; first and fourth segment had dark brownish in color and the second as well third segment found with light brown in color during the laboratory condition studies. The fore legs were smaller and hind legs bigger one. The tibia was longer than other parts of leg (Fig. 5A). The mating and egg lying behavior were also observed under laboratory conditions (Fig. 5C; 5D).

4. Discussion

Dusky cotton bug was remained in the field throughout the year by different host plants other than cotton (Leao et al., 2018). The low temperature and high relative humidity are favorable to

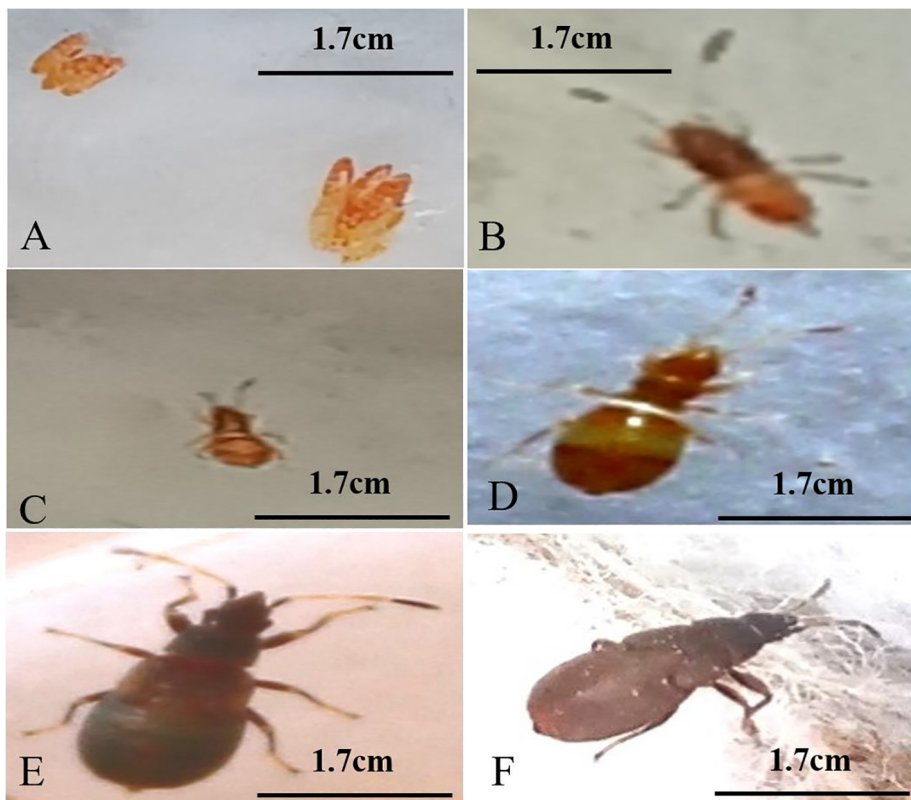


Fig. 4. Different developmental stages of the Dusky cotton bug, Eggs of female DCB (A), first nymph (B), second nymph (C), third nymph (D), fourth nymph (E) and fifth nymph (F).

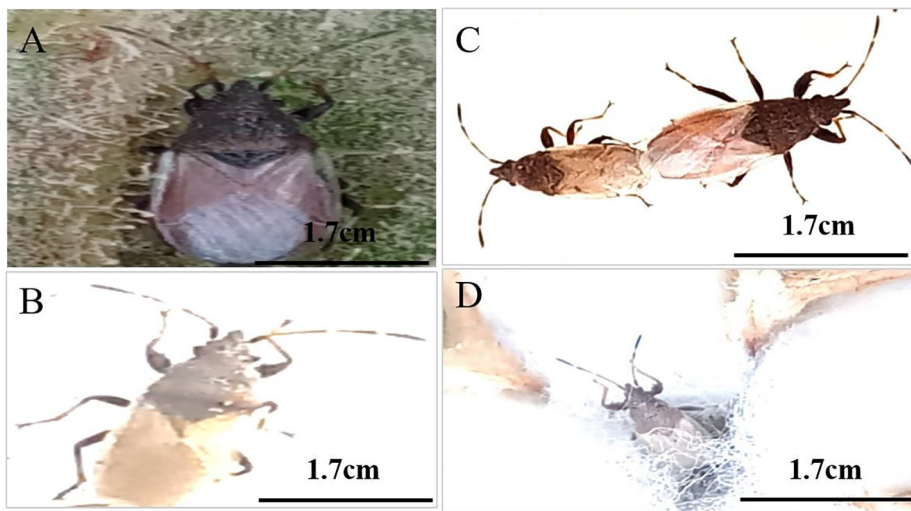


Fig. 5. Different pictures of the DCB, Female DCB bug (A), B; Male DCB bug (B), Mating (C) and DCB in the course of Egg laying (D).

increase the population of Dusky cotton bug (Thangavelu, 1978). Mated couple of DCB was found present on opened bolls of cotton, mostly in morning and evening time. The mating duration of DCB observed significantly different between two seasons *i.e.* winter and summer, in winter, long mating duration recorded as compare to short mating in summer. Whereas, the egg development period, number eggs laid, hatching duration were observed more in winter season as compare to summer. Interestingly, the total number of eggs hatched and percentage of hatchability more observed in summer as compare to winter. The seasonal variations more specifically, the minimum and maximum temperature including

humidity also influence the DCB population but also effects on developmental periods, egg laying, survival percentage as well as hatching percentages of cotton crops (Abbas et al., 2015; Pearson, 1958).

In the past, DCB once declared as a minor pest but recently attained the status of major pest (Shahid et al., 2017). The immature and mature stages of DCB development can damage the cotton crop (Akram et al., 2013). During winter among five nymph stages of DCB, first nymph, soon after hatching was observed moving the antennae in the searching of food up to four days (Molet et al., 2011). The second and third nymph took six to eight days respec-

tively (Fig. 3). While, fourth nymph slightly wing pads appeared on thorax region and last 17 days. Fifth nymph of DCB was with well-developed wing pads with enlarged body and total duration was nine days (Khan and Naveed, 2017; Pearson, 1958; Shahen et al., 2018a), whereas, the female and male developmental period also significantly different in winter and summer (Goergen et al., 2016; Schmutterer, 1969). It has been reported that the nymphs to have different morphologies from first stage up to seven to 15 days at 25–32 °C in Egypt (Molet et al., 2011; Schmutterer, 1969; Shahen et al., 2018b). DCB life cycle was longer in winter as in low temperature and developmental period found the longest in winter season started from the month of November to March.

The faster developmental period was also favored by moist seeds of cotton crop plants for this pest therefore the growth rate and growth efficiency increased as the suitable diet enhancement (Chaplin and Chaplin, 1981). The model of food consumption of *O. laetus* Kirby show that more quantity of food consumed when maintained on seed of *G. hirsutum* than other host plants. It was found that maximum egg production occurred when bugs were continuously maintained on diet of cotton seed (Raman and Sanjayan, 1983). The present results are well fit with Raman and Sanjayan that the seed of *G. hirsutum* was preferred host of *O. laetus* which might be contained the high quantity of nitrogen, proteins, lipids, low carbohydrates and narrow carbon / nitrogen ratio. Further, in literature, has also been reported that the amount of nitrogen increased then the rate of fecundity, longevity and survival rates of phytophagous insects were increased to insect population (Ananthkrishnan et al., 1982; Chaplin and Chaplin, 1981; Raman and Sanjayan, 1983; Shu et al., 2018).

The newly laid eggs of DCB were transparent, spindle and egg-shaped, while the color slowly changed into light yellowish and light orange when near it is near to hatching. First nymph was with small black tail like development at the anal end of the abdomen. The early nymphs were orange in color when near to molting, but slowly turn to reddish brown after molting and became darker after each molt (Vennila et al., 2007a). The newly hatched nymphs were tiny, light reddish brown in color (Kirkpatrick, 1923). The freshly second nymph was orange in color and pro, meso thorax dark brown but *meta*-thorax was light brown in color except head which was dark brown in color. The second nymph was similar with first one head dark brown. The second nymph was with four segmented antennae and last segment was thicker than other segments. Legs were dark brown in color. Our research findings more or less related with (Kirkpatrick, 1923) who reported that the second nymph was similar to the proceeding nymph stage, except the size with dark reddish brown when exposed in air. Furthermore, initially, third stage was light brown and changed to dark brown. It was with broad white line on the thorax which distinguished characters from other stages. Freshly molted third nymph stage was light brown color and slowly turn to dark brown color. (Traver and Edmunds, 1968) who supported that third nymph color was fainter at both the anterior and posterior parts and two white bands appeared on the thorax that character made them different from previous nymphs.

The initial color of fourth nymph was light brown but changed into dark brown up to six hours. The nymph body length was bigger than previous nymph stages with lightly developed wing pads. It was with four segmented antennae with three pair of legs and segmented abdomen. (Kirkpatrick, 1923) also reported that the fourth nymph was wing pads, cover the side margins of the *meta*-thorax were present. The initial color of nymph was light brown then changed to dark brown color. Fully developed wing pads appeared on the thorax and its color was dark brown. White dots appeared on thorax, wing pads and abdomen. The first and fourth segments of antennae dark brown and second and third segment of antennae color were with light brown.

The newly emerged adult was light brown in color. The adult body color turned to dark brown. The adult male was a slender shape abdomen and smaller size than female. It has four segmented antennae and wings were transparent with white dusky hemelytron. The small hair found on head and thorax. It was with three pairs of leg, femur wider and dark brown, while tibia and tarsus were light dusky in color. On the other hand (Vennila et al., 2007a) reported that adults has pointed head; dusky brown with dull white transparent wings and black spots on fore wings with deep red legs (Vennila et al., 2007b). It has also been reported that the adult was a small, elongated bug, pointed head with dark brown or black with dorsally reddish brown abdomen and transparent hemelytra (Pearson, 1958). The antennae have dark brown to black antennae and two-third of the basal segments have light brownish yellow (Rao, 1981).

5. Conclusion and recommendation

In conclusion, the DCB is emerging as a new threat to the cotton crop. But still it needs more effort and research to evaluate further the effects on different crops. In the winter season, though the average number of eggs were 18.93, but the hatching percentage of the eggs were surprising reached up to more than 73 percentage under laboratory conditions. Furthermore, the nymph stage, more specifically, the fourth stage was longer in average days from other nymph stages. Whereas, the fifth nymph stage, wing pads were more developed than fourth stage. In terms of adult longevity, the female life span in average, took more number of days than adult male life span of DCB. The egg color of this menace pest of cotton lint moisture sucking was found from light yellow to orange color. The first nymph stage was tail like development at the anal end of the abdomen which made them distinguished from second stage. The third nymph stage was broad white line emerged on the abdomen which differentiates third nymphs from previous stages. The fourth stage was with fully mature wing pads on the thorax of fifth nymph stage. The newly emerged adult was light brown color. The adult male was a slender shaped abdomen and smaller size than female with four segmented antennae, transparent wings and hemelytron was white dusky with three pair of legs.

The DCB is pest of major cash crops in Pakistan especially cotton crop, it is better to consider the best tools such as geometric morphometry for morphological characters in winter and summer, eco-friendly management by using the Integrated Pest Management (IPM) techniques. In natural conditions, the study of predators could also be under consideration.

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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