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Assessment of density-dependent feeding damage by the cotton dusky bug, *Oxycarenus laetus* Kirby (Hemiptera: Lygaeidae), in cotton

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Abstract: The dusky cotton bug, *Oxycarenus laetus* Kirby (Hemiptera: Lygaeidae), is a serious threat to cotton due to early cultivation of Bt cotton. These studies were carried out under laboratory and field conditions to estimate the feeding damage caused by *O. laetus* to seed weight, seed germination, and lint quality of cotton cultivar Bt-121 at 6 bug densities (0, 5, 10, 15, 20, and 25 adult pairs). The results revealed that seed germination and seed weight decreased, whereas percent reduction in seed germination and seed weight over control increased with increasing bug density. Bug density in the range of 5–25 pairs caused approximately 9.3%–40.7% reduction in seed germination and 7.5%–29.5% reduction in 100-seed weight over control in both laboratory and field trails. The color of lint changed from pure-white to white when bolls were exposed to 10 and 15 pairs of bugs, while it changed to light-yellow and slightly yellow when bolls were exposed to 20 and 25 pairs of bugs, respectively. Bug density had a high negative correlation with percent seed germination and 100-seed weight, while it had a high positive correlation with reduction in seed germination, reduction in 100-seed weight, and lint quality, under both laboratory and field conditions. Bug density explained more than 95% of total variability in seed germination, reduction in seed germination, 100-seed weight, and reduction in 100-seed weight under both laboratory and field conditions; it explained less than 95% (93.2%) of total variability in lint staining under field conditions. These results indicate that reductions in seed weight, seed germination, and lint quality depend upon bug density. Hence, appropriate control measures should be adopted to check consistent increases in the dusky cotton bug population to avoid seed germination and lint quality losses.

Key words: Dusky cotton bug, population densities, lint quality, seed germination, cotton

1. Introduction

Cotton is an important cash crop for Pakistan and a significant source of foreign exchange earning. It accounts for 7.8% of the value added in agriculture and 1.6% of the economy's gross domestic product (Farooq, 2012). The value added by the cotton crop accounts for 37.1% of the overall agriculture earnings (Farooq, 2011). It produces not only the most important textile fiber for textile mills and garment factories, but also contributes in the domestic production of edible oil, while cotton cake is fed to dairy cattle. Amongst various factors responsible for its low yield, insect pest outbreaks are of paramount importance, causing heavy (20% to 40%) yield losses in Pakistan (Aslam et al., 2004; FAO, 2007).

Cotton in Pakistan has to face attack from sucking as well as chewing insect pests every year. However, with the advent of Bt cotton and changes in the sowing routine of cotton crops, pest status has completely changed. Higher incidence of minor insect pests like the dusky cotton bug and stink bug on Bt cotton (Patil and Rajanikanth, 2005; Patil et al., 2006) is becoming a great threat to cotton in Pakistan. The dusky cotton bug is flourishing and multiplying rapidly. This bug appeared on cotton in May 2010 where Bt cotton was sown in the month of February (early sowing) and caused premature falling of flowers, squares/brackets, and small bolls. The dusky cotton bug is expected to gain the status of pest in Pakistan because it has the status of a major pest of cotton, causing qualitative and quantitative losses to cotton in many areas/countries. Henry (1983) reported dusky cotton bug as a serious cotton pest in Egypt, where it has caused weight loss in cottonseed and decreased germination and oil quality of the seed. Hill (1983) reported it as a major pest in Southeast Asia, India, and Africa on both cotton and okra. In Israel, the dusky cotton bug has caused tree fruit damage, including greasy spots caused by adults sucking on fruits and disfigurement of the fruits (Avidov and Harpaz, 1969) due to its toxic saliva (Schaefer and Panizzi, 2000). It primarily feeds on seeds of plants in the family Malvaceae, particularly *Gossypium* spp. (cotton); however, in addition to cotton,

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this pest has also been intercepted on certain fruits and vegetables including apple, avocado, corn, dates, fig, grapes, peach, okra, pineapple, and pomegranate, as well as on hibiscus (USDA, 2009, 2010). Dusky cotton bug causes considerable reduction in weight of seeds, their viability, and their oil content (Peacock, 1913; Balls, 1915; Misra, 1921; Kirkpatrick, 1923; Pearson, 1958; Ananthakrishan et al., 1982; Hill, 1983; Rajashekhargouda et al., 1983; Schaefer and Panizzi, 2000). Sewify and Semeadaj (1993) reported 6.8%, 32%, and 6% reduction in cotton yield, seed weight, and oil content, respectively. According to Srinivas and Patil (2004), the dusky cotton bug caused 42.9%, 40.8%, 35.1%, and 29.3% losses in seed cotton weight, seed weight, oil content, and seed germination, respectively, when the population was 50 pairs per boll. Lint of cotton is stained pinkish from the crushed insects (Henry, 1983). Although cotton seeds appear normal from the outside, the embryos are shriveled and discolored (Kirkpatrick, 1923), and weight loss can occur up to 15% (Schaefer and Panizzi, 2000).

In Pakistan, local cultivars have been genetically modified and engineered with Bt-toxin. Before the cultivation of Bt cotton, conventional cotton was cultivated in May and harvested in October or November, and the dusky cotton bug was considered to be a minor pest as it appeared very late at the boll maturation stage, particularly at the last 2 pickings. That is why no or very little research has been conducted on the estimation of losses caused by this bug. However, attacks and damage at the early reproductive stage on very-early–sown Bt cotton in the Cotton Belt has spurred a need to estimate bug-density–dependent losses caused by the dusky cotton bug. These studies were carried out under laboratory as well as field conditions to determine seed weight, seed germeability/viability, and lint quality losses caused by different densities of the bug.

2. Materials and methods

2.1. Insect source

Adults of *O. laetus* (males and females) were aspirated from the laboratory culture maintained at 28 ± 1 °C, 65 ± 5% rh, and 12:12 D:L period on soaked seeds of cotton in the insect-rearing laboratory of the Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad.

2.2. Laboratory studies

Seed of cotton cultivar Bt-121 were collected from cotton fields maintained at the cotton research area of the Cotton Research Institute, Ayub Agricultural Research Institute, Faisalabad. These fields were kept protected from attack from sucking as well as chewing insect pests by intensive spraying of insecticides, as these fields were maintained for seed production. The collected seeds were brought to the laboratory of the Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad. These seeds were delinted with HCl and then washed with water 3 times to remove the acid from the seeds. Then 100 g of seed was weighed with an electronic weighing balance and placed in a petri dish that had already been sterilized. Twenty-four such petri dishes were prepared, each having 100 g of seed. These petri dishes were divided into 6 sets, each having 4 petri dishes that served as 4 replications. Six densities of *O. laetus* (0, 5, 10, 15, 20, and 25 pairs of bugs per replicate) were used in the experiment. The required number of pairs (newly emerged adult males and females at a 1:1 ratio) were aspirated from the laboratory culture and released on 100 g of seed. The experiment was performed under controlled laboratory conditions (25 ± 1 °C and 45 ± 5% rh) in a completely randomized design (CRD). The exposed seeds were weighed after exposure of 72 h, and percent reduction in seed weight over the control was calculated by the following formula (1):

$$\text{Weight reduction over control (\%)} = \frac{WT_b - WT_a}{WC_b - WC_a} \times 100 \quad (1)$$

WT_b = weight 72 h before treatment in treated set;
WT_a = weight 72 h after treatment in treated set;
WC_b = weight 72 h before in control set;
WC_a = weight 72 h after in control set

Then 100 g of seed, exposed to different densities of bugs, was counted and separately placed on water soaked paper towel for germination as described by Srinivas and Patil (2004). The number of germinated seeds was counted and percent seed germination was calculated by the following formula (2):

$$\text{Seed germination (\%)} = \frac{G_s}{T_s} \times 100 \quad (2)$$

T_s = Total number of seeds;
G_s = Number of geminating seeds

Then percentage seed germination reduction over the control was calculated by the following formula (3):

$$\text{Percent seed germination reduction over control (\%)} = \frac{SG_c - SG_t}{SG_c} \times 100 \quad (3)$$

SG_c = Number of seeds germinated in control set;
SG_t = Number of seeds germinated in treated set

2.3. Field studies

A lot of 100 g of seed of cotton cultivar Bt-121 was exposed to 6 densities of dusky cotton bug, as described in the laboratory studies section (Section 2.2). Twenty-four such lots were prepared and divided into 6 sets each having 4 lots. The seeds thus exposed to different bug densities were sown in the field in a randomized complete block design with 4 replications during 2010. Each treatment plot measured 7 m × 3 m, having 5 cotton rows, and plant (seed) and row spacing were 30 and 60 cm, respectively. Twenty-three seeds were planted in each row and germinated seeds/plants were counted. Then, percent seed germination as well as percent seed germination reduction over control was calculated by the formulae given in the laboratory studies section. The same experiment was used for investigating the effects of bug density on lint quality. The experimental plots were kept free from attack from sucking and bollworm insects by spraying systemic (Buprofezin 25% WP, Imidacloprid 20% SL, and Nitenpyram 10SL, alternatively) and stomach (Emamectin benzoate 1.9 EC, Leufenuron 5% EC, and Profenofos 500 EC, alternatively) poison. On their appearance and before the opening of bolls on the cotton plants, 10 healthy bolls of almost uniform size were randomly selected per plant. Each boll was observed under a magnifying hand lens for any infestation of pink boll. Each of 5 bug densities (5, 10, 15, 20, and 25 pairs [newly emerged adult males and females in 1:1]) of *O. laetus* was maintained on 2 of these 10 bolls separately, with the help of specially prepared small muslin cloth bags to avoid any escape of the retained bug population or natural contamination by other sucking insects. After observation, and ensuring under magnifying hand lens that there was no pink bollworm infestation, 2 healthy bolls were bagged on each plant as control treatment where no bug was retained. In each plot, 20 plants were treated likewise.

At the boll maturation stage, the color of the lint picked from each boll of each respective treatment was visually observed and graded as per scales for determination of visual staining of lint (Table 1). The seeds were separated manually from the lint of each respective treatment and placed in separate trays. From the collected seeds of each treatment, 100 seeds were taken and weighed by electronic weighing machine. Then the percent reduction in 100-seed weight over the control was calculated by the following formula (4):

$$\text{Percent reduction in 100-seed weight over control (\%)} = \frac{SW_c - SW_t}{SW_c} \times 10 \quad (4)$$

SW_c = 100-seed weight in control set;
SW_t = 100-seed weight in treated set

Table 1. Scale for determination of visual staining of lint caused by the dusky cotton bug.

Lint staining scale	Color of lint
0	Pure white
≤1	White
≤2	Light yellow
≤3	Slightly yellow
≤4	Slightly dark yellow
≤5	Dark yellow

2.4. Statistical analysis

The data regarding dependent variables including seed germination, seed weight, percent reduction in seed germination and seed weight over control, and color of lint were subjected to ANOVA at a probability level of 5%, by using SPSS (O'Connor, 2000) to determine the parameters of significance and means value for different treatments (bug densities). The means of significant treatments were compared with Tukey's honestly significant difference (HSD) (Danho et al., 2002). Regression between all aforementioned dependent parameters and bug density was also established, using linear regression and Pearson correlation analysis at 5% level of probability.

3. Results

3.1. Impact of dusky cotton bug on seed germination under laboratory and field conditions

Dusky cotton bug population had significant effect on seed germination in field and laboratory conditions ($P < 0.05$) (Table 2). A significant variation was observed in the percent germination as well as in the percent reduction in germination over control under laboratory and field conditions (Figures 1 and 2). A density-dependent variation was observed in percent germination and percent reduction in germination over the control. Percent germination decreased and percent reduction in germination over the control increased significantly with an increase in the density of dusky cotton bug under both laboratory and field conditions (Figures 1 and 2). The percent germination ranged from 64.9% to 83.9% and 64.2% to 81.8% in the laboratory and field conditions, respectively, being significantly higher with higher and lower with lower bug density (Figure 1). The percent reduction in germination over the control ranged from 8.5% to 27.6% and 11.2% to 28.8% in laboratory and field conditions, respectively, being significantly lower with higher and higher with lower bug density (Figure 2). The results revealed that bug density in the range of 5–25 pairs

Table 2. ANOVA parameters of different bug's densities for seed germination, percent reduction in seed germination, 100-seed weight, percent reduction in 100-seed weight, and lint staining of cotton.

Dependent variable	df	Bug densities (independent variable)			
		Laboratory conditions		Field conditions	
		F	P < 0.05	F	P < 0.05
Seed germination	5[a]/15[b]	392.7	0.0001	406.6	0.0003
Percent reduction in seed germination	5[a]/15[b]	99.6	0.001	103.7	0.003
100-seed weight	5[a]/15[b]	888.7	0.00001	486.6	0.0001
Percent reduction in 100-seed weight	5[a]/15[b]	1025.1	0.00002	1130.5	0.0002
Lint staining	5[a]/15[b]	-	-	115.3	0.001

[a] = Degree of freedom for bug densities; [b] = Error degree of freedom.

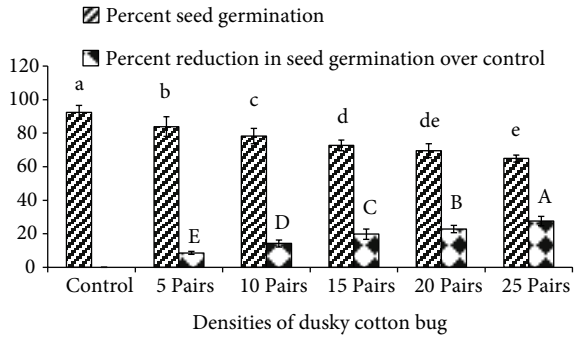

Figure 1. Seed germination (%) and reduction in seed germination over control (%) when seed were exposed to different densities of *Oxycarenus laetus* under laboratory conditions.

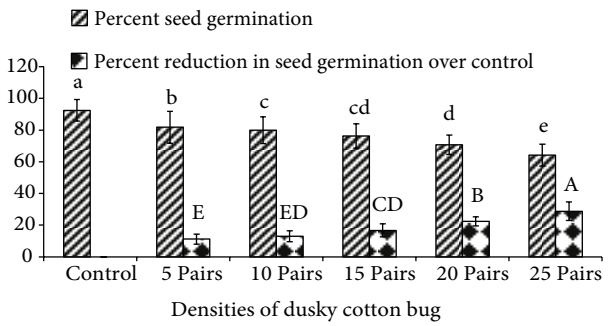

Figure 2. Seed germination (%) and reduction in seed germination over control (%) under field conditions when seeds were exposed to different densities of *Oxycarenus laetus*.

caused approximately 9%–40% reduction in cottonseed germination over control, where no bug was released (Figures 1 and 2).

3.2. Impact of dusky cotton bug on 100-seed weight under laboratory conditions

Different densities of dusky cotton bug had significant effects on 100-seed weight and percentage reduction in 100-seed weight under laboratory conditions (P < 0.05) (Table 2). The 100-seed weight was in the range of 6.6–7.4 g, being significantly higher at lower and lower at higher bug density. The biotic stress of various bug densities (5–25 pairs) caused a 7.3%–18% reduction in the weight of 100 seeds over the control (where no bug was released) (Figure 3).

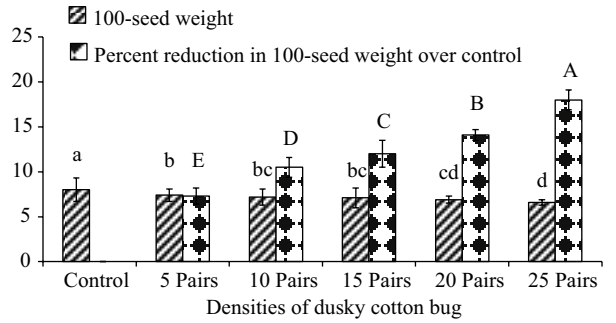

Figure 3. 100-seed weight (g) and reduction in 100-seed weight over control (%) when seeds were exposed to different densities of *Oxycarenus laetus* under laboratory conditions.

3.3. Impact of dusky cotton bug on 100-seed weight under field conditions

Different densities of dusky cotton bug had significant effect on 100-seed weight and percentage reduction in 100-seed weight under field conditions (P < 0.05) (Table 2). The 100-seed weight was less in bug-treated seeds as compared to untreated 100-seed lots (16.5 g), and ranged from 9.4 to 16.5 g. The 100-seed weight decreased with an increase in the density of dusky cotton bug. Similarly, percent reduction in 100-seed weight over control ranged from 9.6% to 29.5% and increased with an increase in the density of dusky cotton bug (Figure 4).

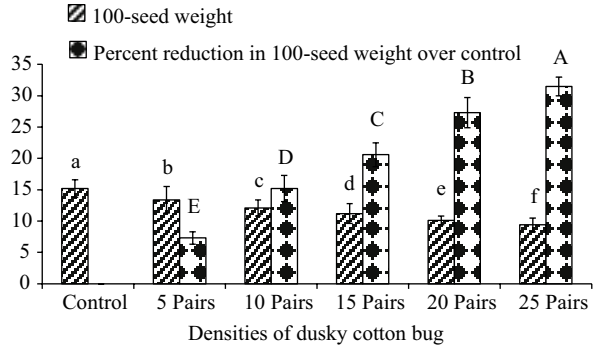

Figure 4. 100-seed weight (g) and reduction in 100-seed weight over control (%) when bolls were exposed to different densities of *Oxycarenus laetus* under field conditions.

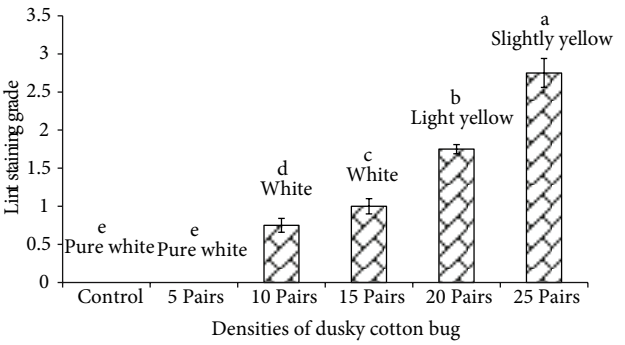

Figure 5. Lint staining grades and color change when bolls were exposed to different densities of *Oxycarenus laetus* under field conditions.

3.4. Impact of dusky cotton bug on lint staining

Significant variation in lint color was observed when exposed to different bug densities (Table 2; Figure 5). The lint exposed to no and 5 pairs of bugs revealed no change in its color and remained pure white (zero grade staining). According to the lint grade scanning scale (Table 1), the color of lint changed to white (0.75 grade staining), white (1.0 grade staining), light yellow (1.75 grade staining), and slightly yellow (2.75 grade staining) when bolls were exposed to 10, 15, 20, and 25 pairs of bugs, respectively (Figure 5).

3.5. Regression and correlation between yield parameters and bug densities

The probability value ($P < 0.05$) shows that a relationship existed between bug density and all evaluated dependent variables for laboratory and field studies (Table 3). The correlation coefficient values (r) (Table 3) and scatter diagrams (Figures 6 and 7) reveal that bug density had a high negative correlation with percent seed germination and 100-seed weight in laboratory and field conditions as coefficient of correlation values were very close to negative one (–1) value (Table 3), and data points were found scattered very close to a negatively sloped line (Figures 6 and 7). However, reduction in seed germination and reduction in 100-seed weight as well as visual staining of lint had a high positive correlation with the bug densities, because their coefficient of correlation values were very close to positive one (+1) value (Table 3), and data points were found scattered very close to a positively sloped line (Figures 6 and 7).

Regression parameters and scatter diagrams reveal that density of dusky cotton bug had a significant linear relationship with and explained significant variability in seed germination, reduction in seed germination, 100-seed weight, reduction in seed weight, and color of lint ($P < 0.05$) (Table 4; Figures 6 and 7). Under laboratory conditions, 97.04%, 97.61%, 99.0%, and 98.91% of total variability in 100-seed weight, reduction in 100-seed weight, seed

Table 3. Correlation coefficients values (r) for seed germination, percent reduction in seed germination, 100-seed weight, percent reduction in 100-seed weight, and lint staining against bug density.

Dependent variables	Coefficient of correlation values	
	Laboratory r ($P < 0.05$)	Field r ($P < 0.05$)
Seed germination	–0.988 (0.001)	–0.978 (0.001)
Percent reduction in seed germination	+0.987 (0.0001)	+0.978 (0.001)
100-seed weight	–0.985 (0.000)	–0.946 (0.001)
Percent reduction in 100-seed weight	+0.984 (0.000)	+0.965 (0.000)
Lint staining	-	+0.966 (0.001)

r = Coefficient of correlation value.

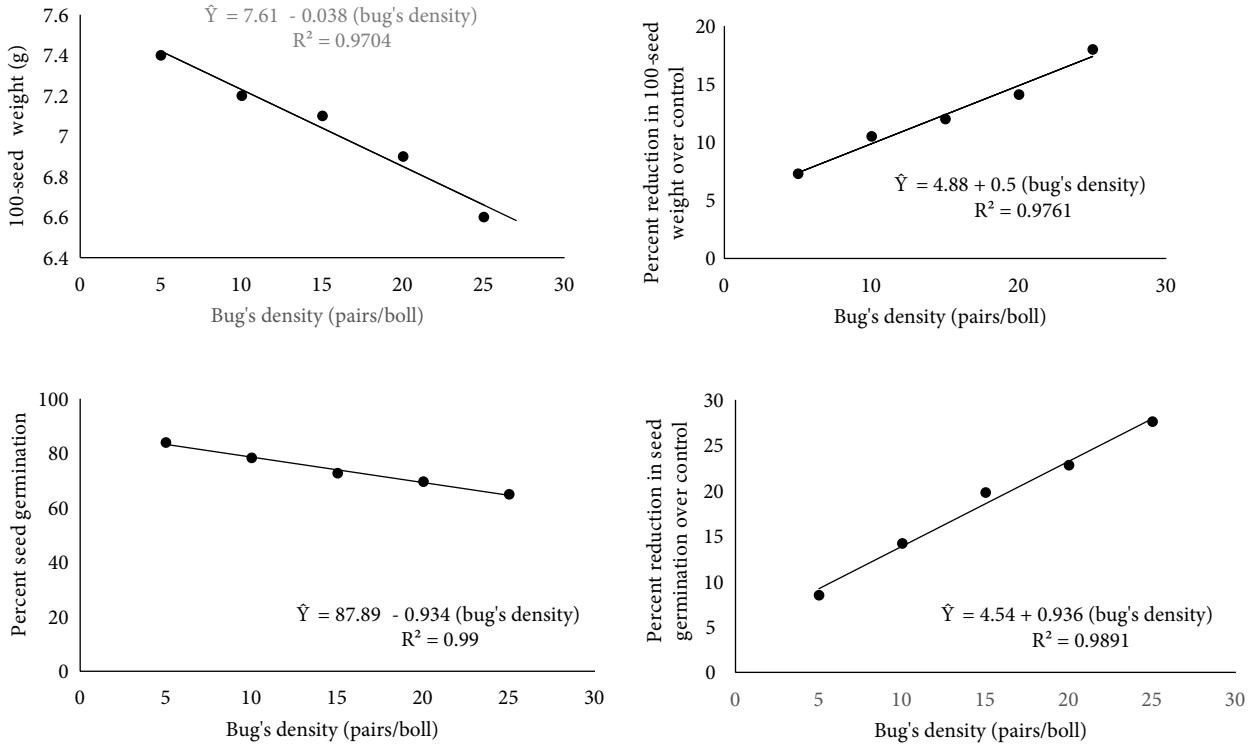

Figure 6. Coefficient of determination (R^2), linear regression equation, and scatter diagram showing the fitted simple regression line of Ŷ (100-seed weight, percent reduction in 100-seed weight, seed germination, and percent reduction in seed germination) on X (densities of *Oxycarenus laetus*) under laboratory conditions.

germination, and reduction in seed germination over the control, respectively, was explained by bug density (Table 4; Figure 6). Under field conditions, bug density explained 99.0%, 99.0%, 95.6%, 95.5%, and 93.2% of total variability in 100-seed weight, reduction in 100-seed weight, seed germination, reduction in seed germination over control, and lint color, respectively (Table 4; Figure 7).

4. Discussion

With the early cultivation of Bt cotton on large areas and a drastic reduction in insecticide application against bollworms in the Cotton Belt, the dusky cotton bug, which had been considered a minor pest, started causing damage to reproductive parts of the cotton plant at a very early stage. The results of the present research also reveal the bugs' damage to bolls, seed germination, seed weight, and lint color. These studies included laboratory and field evaluation of bug-density–dependent damage to seed weight, seed germination, and lint quality. The seeds exposed to different densities of bug showed significant decreases in seed weight and seed germination with an increase in bug density. These results are compatible with those of Peacock (1913), Balls (1915), Misra (1921), Pearson (1958), Ananthakrishan et al. (1982), Hill (1983), Rajashekhargouda et al. (1983), and Schaefer and Panizzi (2000), who have reported considerable reductions in weight of seeds, viability, and oil content due to attacks by the dusky cotton bug. These results are also highly in agreement with those of Henry (1983), who reported dusky cotton bug as a serious cotton pest in Egypt causing weight loss in cottonseed, reduced germination, and decreased oil quality of the seed. The results of the present study reveal that the dusky cotton bug has the potential for causing serious damage to cotton in terms of cottonseed weight and cotton lint. Hill (1983) also reported it as a major pest in southeast Asia, India, and Africa on both cotton and okra. Sewify and Semeadaj (1993) reported 6.8%, 32%, and 6% reduction in cotton yield, seed weight, and oil content, respectively. According to Srinivas and Patil (2004), the dusky cotton bug caused 42.9%, 40.8%, 35.2%, and 29%–32% losses in seed cotton weight, seed weight, oil contents, and seed germination, respectively, when the population was 50 pairs per boll (maximum density). These results also confirm the findings of the present study, which revealed that bug density in the range of 5–25 pairs caused approximately 9.3%–40.7% reduction in seed germination and 7.5%–29.5% reduction in 100-seed weight over the control (where no bugs were released) in both laboratory and field trials. The reduction in seed weight is attributed to its feeding on the oil contents of seed as documented

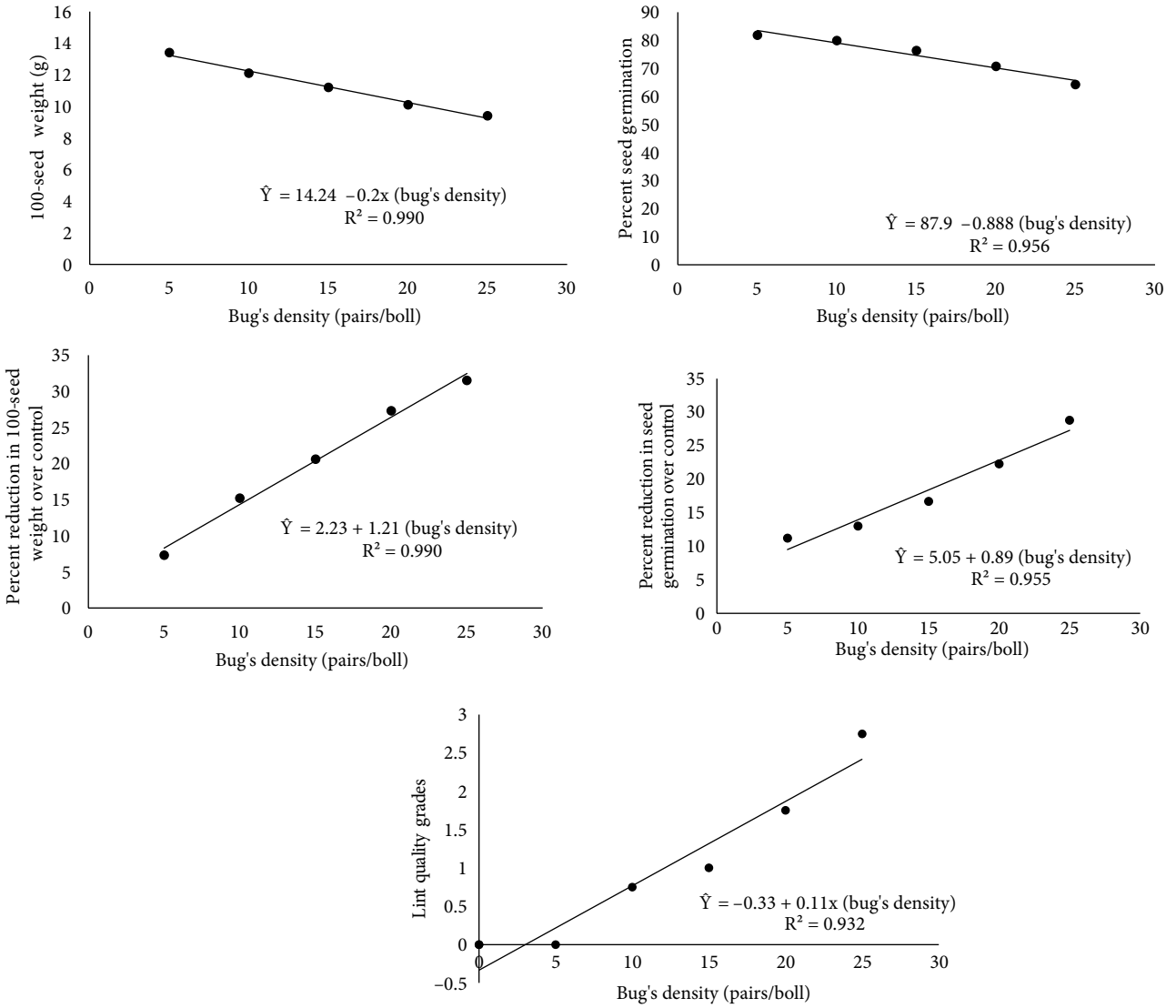

Figure 7. Coefficient of determination (R^2), linear regression equation, and scatter diagram showing the fitted simple regression line of Ŷ (100-seed weight, percent reduction in 100-seed weight, seed germination, percent reduction in seed germination, and lint quality grades) on X (densities of *Oxycarenus laetus*) under field conditions.

in a report compiled by the USDA (2009, 2010), whereas reduction in seed germination may be attributed to severe shriveling of the embryos inside the seeds, which appear normal externally (Kirkpatrick, 1923). According to the lint scanning scale used, the color of lint changed from pure-white to white when bolls were exposed to 10 and 15 pairs of bugs, whereas the color changed to light yellow and slightly yellow when bolls were exposed to 20 and 25 pairs of bugs, respectively. These results are not in agreement with those of Henry (1983), who has reported that lint of cotton is stained pinkish from crushed insects. This variation in result may be attributed to the fact that he observed the color of lint after crushing the insects, while the color of lint in the present study was observed after feeding or damage by the adult bugs. The yellow coloration may be due to the reaction of toxic saliva, secreted during feeding, with immature cotton lint as reported by Schaefer and Panizzi (2000). The bug density had a high negative correlation with percent of seed germination and 100-seed weight in laboratory and field conditions; reduction in seed germination and reduction in 100-seed weight as well as visual staining of lint had a high positive correlation with the bug densities. The bug density explained more than 95% of total variability in seed germination, reduction in seed germination, 100-seed weight, and reduction in 100-seed weight under both laboratory and

Table 4. ANOVA parameters and coefficient of determination of linear regression equations for seed germination, percent reduction in seed germination, 100-seed weight, percent reduction in 100-seed weight, and lint staining in relation to bug density.

Yield parameters	Bug densities (independent variable)			
	df	F value	(P < 0.05)	100 R^2 (%)
Under laboratory conditions				
Percent seed germination	$1^a/3^b$	297.5	0.000	97.04
Percent reduction in seed germination	$1^a/3^b$	271.1	0.000	97.61
100-seed weight (g)	$1^a/3^b$	98.5	0.002	99.00
Percent reduction in 100-seed weight	$1^a/3^b$	122.7	0.002	98.91
Under field conditions				
Percent seed germination	$1^a/3^b$	63.3	0.004	99.00
Percent reduction in seed germination	$1^a/3^b$	64.3	0.005	99.00
100-seed weight (g)	$1^a/3^b$	326.1	0.000	95.60
Percent reduction in 100-seed weight	$1^a/3^b$	315.3	0.000	95.50
Visual staining of lint	$1^a/3^b$	84.5	0.003	93.20

df = Degree of freedom; ^{a} = Degree of freedom for bug densities; ^{b} = Error degree of freedom; R^2 = Coefficient of determination.

field conditions. Similarly, less than 95% (93.2%) of total variability in lint staining was explained by bug density under field conditions. These results cannot be confirmed or contradicted because information regarding these results was not found in the reviewed literature. These results indicate that loss in seed weight and germination and deterioration of lint quality depend upon bug density. Further increases in bug density may cause more severe losses in seed germeability/viability and lint quality at initial as well as at later reproductive growth stages of cotton plants. Hence, appropriate control measures should be adopted to check regular increases in the dusky cotton bug population, to avoid seed germination and lint quality losses.

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