



Original article

Seasonal population dynamics of dusky cotton bug (*Oxycarenus* spp.) in transgenic cotton varieties under field conditionsJavaid Iqbal^{a,b,c}, Shahzad Ahmad Bhutta^c, Abdulaziz S. Alqarni^{a,*}, Ayman A. Owayss^a, Mohammad Javed Ansari^d^a Melittology Research Lab., Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia^b Department of Entomology, MNS University of Agriculture Multan, Pakistan^c Department of Entomology, University College of Agriculture & Environmental Sciences, The Islamia University of Bahawalpur, Pakistan^d Bee Research Chair, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

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ABSTRACT

Dusky cotton bug (*Oxycarenus* spp.) has become a major insect pest for cotton crop in Pakistan. Transgenic cotton varieties provided resistance to a variety of insects pests. But, these are not safe for this emerging potential threat. In present study, nine transgenic cotton varieties (IUB-222, MNH-886, FH-142, CIM-599, A-555, CIM-602, NIAB-777, MNH-786 and Bt-666) were assessed for seasonal population dynamics of dusky cotton bug (DCB) under field conditions. All transgenic varieties showed a differential DCB population over the months and no transgenic variety was free from DCB population throughout the crop duration. DCB population appeared during 3rd week of July and crossed the economic threshold level (10–15 nymph/ adults or both per plant) during August. A substantial increase in DCB population was noted during September–November with its peak population during October, 2014. Among all varieties, three varieties (CIM-599, CIM-602 & IUB-222) showed a significantly lower mean population per plant (37.76, 37.87, 43.84) and two varieties (FH-142, MNH-886) gave highest population (44.71, 46.81), respectively. Correlation matrix revealed that low temperature and high humidity were promoting the DCB population. Cluster analysis revealed interesting findings that IUB-222 with least population fall in a cluster where other two varieties (FH-142 & MNH-886) possessed highest population. Moreover, two varieties (CIM-599 & CIM-602) with least population fall in second cluster regarding DCB population. These findings would be helpful for the farmers to select the varieties that showed relatively higher resistance towards DCB population and to adopt proper management strategies keeping in view the trend of DCB population during the crop season.

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1. Introduction

Cotton has been an integral part of human agriculture for hundreds of decades. It has mainstay in the economy of Pakistan as it provides fiber as export items, raw material for textile, edible oil and animal food (Ozyigit et al., 2007). Pakistan stands at 4th position in the world regarding cotton production after China, India and USA (Iqbal et al., 2010). In terms of area, cotton is the second

largest grown crop in Pakistan after wheat that contributes 1.0% in gross domestic product (GDP) and 5.1% value addition in agriculture (GOP, 2016). A declined rate –27.83% in cotton production was observed with 10.1 million cotton bales during 2015–16 as compared to last year's production 13.96 million bales (GOP, 2016). The average per hectare yield of cotton still stands below average due to water scarcity, improper fertilizer management, weed invasion, disease attack and above all severe insect pest infestation (Ahmed et al., 2009; Arshad and Suhail, 2010; USDA, 2016; Rehman et al., 2017).

Conventional cotton varieties (*Gossypium* spp.) are cultivated commercially all over the world but there is an exponential increase in the cultivation of genetically modified varieties in past few years. USA was the first country to test Bt protected cotton crop in the field during 1990 and later it became one of the most extensively used genetically engineered crops in the world (Qaim

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and Zilberman, 2003). Transgenic cotton got much unprecedented popularity for its resistance against lepidopterous insect pests especially cotton bollworms (Peshin et al., 2007; Arshad et al., 2011). It also provides high quality fiber regarding fiber length and strength (Ahsan and Altaf, 2009). Its cultivation provided significant benefits in terms of reduced production cost, insecticide application, environmental contamination and increased yield (James, 2002; Dhillon et al., 2011).

Bt cotton contains a gene of *Bacillus thuringiensis* which produces Cry-protein. The ingestion of Cry-toxin by insect larvae resulted in midgut paralysis, disturbed permeability and collapse of epithelium. The larval feeding becomes impossible and after direct contact, its death occurs within 48–72 h (Gill et al., 1992). However, Bt cotton remained unable to provide resistance against many sucking insect pests (Hofs et al., 2004).

In recent years, dusky cotton bug (*Oxycarenus* spp.) got attention for its severe attack in ample number on cotton crop. It is commonly known as cotton stainer or seed cotton bug (Akin et al., 2010; Sammaiah et al., 2012). Dusky cotton bugs (DCB) are crushed during ginning process that stain valuable cotton lint and stained lint fetch low price in the market. The advent of Bt-cotton with changed sowing plan (early sowing) and limited insecticidal applications against bollworms have provided favorable environment for minor pest like DCB to become the major threat of economic importance in Pakistan (Ullah et al., 2016; Shahid et al., 2017).

Considerable qualitative and quantitative losses in cotton are reported after severe DCB infestation (Srinivas and Patil, 2004a; Ahmed et al., 2015). Both adult and nymph of DCB suck fluid from leaves of young stems and oil from mature seeds (Ananthakrishnan et al., 1982; Khan and Ahmed, 2000; Vennila et al., 2007; Akram et al., 2013). Adult DCB has black thorax, translucent white wings and is 4–4.3 mm long. Its female lays up to 110 eggs. There are 6–7 generation per year and its life span ranges from 33–49 days (male) & 35–51 days (female) under controlled conditions (Srinivas and Patil, 2004c; Abbas et al., 2015). The eggs are laid on mature seeds of the host plants in single or in group form. The nymph is pink in color with orange abdomen (Smith and Brambila, 2008). It causes multiple injuries that includes reduction in oil content of seed, seed weight and subsequently reduction in cotton yield. Its infestation leads to injured embryo (shriveled and underdeveloped) and reduces feasibility of seed germination (Sweet, 2000; Srinivas and Patil, 2004a). It exhibits indigenous migratory behavior among host plants throughout the whole year (Holtz, 2006). The final generation overwinter on branches of weeds, leaves, and grasses or other such places. The insect avoids mating, during this period until the availability of host plants (Schaefer and Panizzi, 2000). DCB is a polyphagous insect feeding mainly on plants belonging to family Malvaceae (especially *Gossypium* spp) and also on avocado, corn apple, dates, grapes, peach, okra, pineapple, pomegranate and Figs. (USDA, 2010). In Pakistan, it persists throughout the year and can survive on guava, moringa, mango, lemon, okra, chillies and cotton (Shah et al., 2016).

Since, Bt cotton is extensively cultivated crop in the country. It is worth to monitor their response against such drastic and polyphagous insect keeping in view its severity to cotton lint and seed. Therefore, the present experiment was designed to monitor the seasonal population dynamics in field cultivated Bt-cotton varieties in relation to local climatic conditions. The findings will help the farmer community in problem orientation and its solution.

2. Materials and methods

2.1. Crop cultivation and land preparation

Field experiment was designed to monitor the seasonal population dynamics of DCB on Bt-cotton varieties. Nine varieties

(IUB-222, MNH-886, FH-142, CIM-599, A-555, CIM-602, NIAB-777, MNH-786, Bt-666) were sown during 2014 under Randomized Complete Block Design (RCBD) with three replications at the entomological research area, The Islamia University of Bahawalpur (29.37°N, 71.77°E). Variation in DCB population over time represented the response of different transgenic cotton varieties. The field was divided into 27 plots with plot size 5 m × 3 m and distance between plots was 1 m. The crop was sown by dibbling method during 2nd week of June, 2014. The plant-plant distance was 22.5 cm and row-row distance was maintained at 75 cm. After emergence of crop, manual weeding was practiced to avoid any competition for space, water, nutrients and light with the crop. Standard agronomic practices (fertilizer and irrigation) were applied throughout the season as pre requirement of the crop and no plant protection measures were adopted to control the DCB.

2.2. Data recording and statistical analysis

DCB population was visually recorded by counting the number of adults and nymphs on bolls of five randomly selected plants per plot. The data were recorded in early morning (Sanghi et al., 2014) at weekly interval during whole duration of the crop till harvesting (Qayyum et al., 2014). The mean insect population was used as an indicator for analyzing the varieties response to DCB population. Meteorological data (temperature, humidity and rainfall data) were obtained from Pakistan meteorological department, Islamabad. Correlation were calculated to investigate the possible influence of weather parameters on population of DCB. Cluster diagram was constructed with Past 3 data analysis software from monthly mean DCB population. Statistix 8.1 software was used to analyze the data with analysis of variance (ANOVA) and least significance (LSD) test at 5% probability level.

3. Results and discussion

Results revealed a significant difference in DCB population among transgenic cotton varieties. It was also observed that no variety was free from DCB throughout the crop season and depicted the severity level of this insect pest for lint damage. The weekly population data showed that DCB did not appear during June and 1st–2nd weeks of July (Fig. 1a). Its population started to appear during 3rd week of July and remained below threshold level in August (Fig. 1b). It increased substantially during September (Fig. 1c) by crossing threshold level (10–15 nymph/adults or both per plant) (Anonymous, 2013, 2015) and reached at peak during October–November (Fig. 1d and e). Mean monthly population is depicted in Fig. 2 that clearly indicated the increasing trend from September–November. Shah et al. (2016) also reported the confined activity of DCB during August–November which is in agreement with our findings.

Table 1 indicated the significant level of mean DCB population per month among transgenic cotton varieties. During July–August, DCB population remained below threshold level. From September, DCB population drastically started to increase and MNH-786 revealed highest mean population (35.88). Whereas, minimum population was observed in CIM-602 (23.98) and A-555 (24.27) during September. Threefold increase in DCB population was observed during October on all cotton varieties. FH-142 gave maximum population (108.30) and minimum population was in CIM-599 and NIAB-786 with values (65.88, 69.70), respectively. Likewise, all cotton varieties were also harbored with intensive DCB population during November. NIAB-777 (96.23) and MNH-786 (96.10) gave maximum population and lower population was recorded in Bt-666 (80.30) during November. It is evident that high DCB population (25 pairs) can accelerate drastic damage to cotton

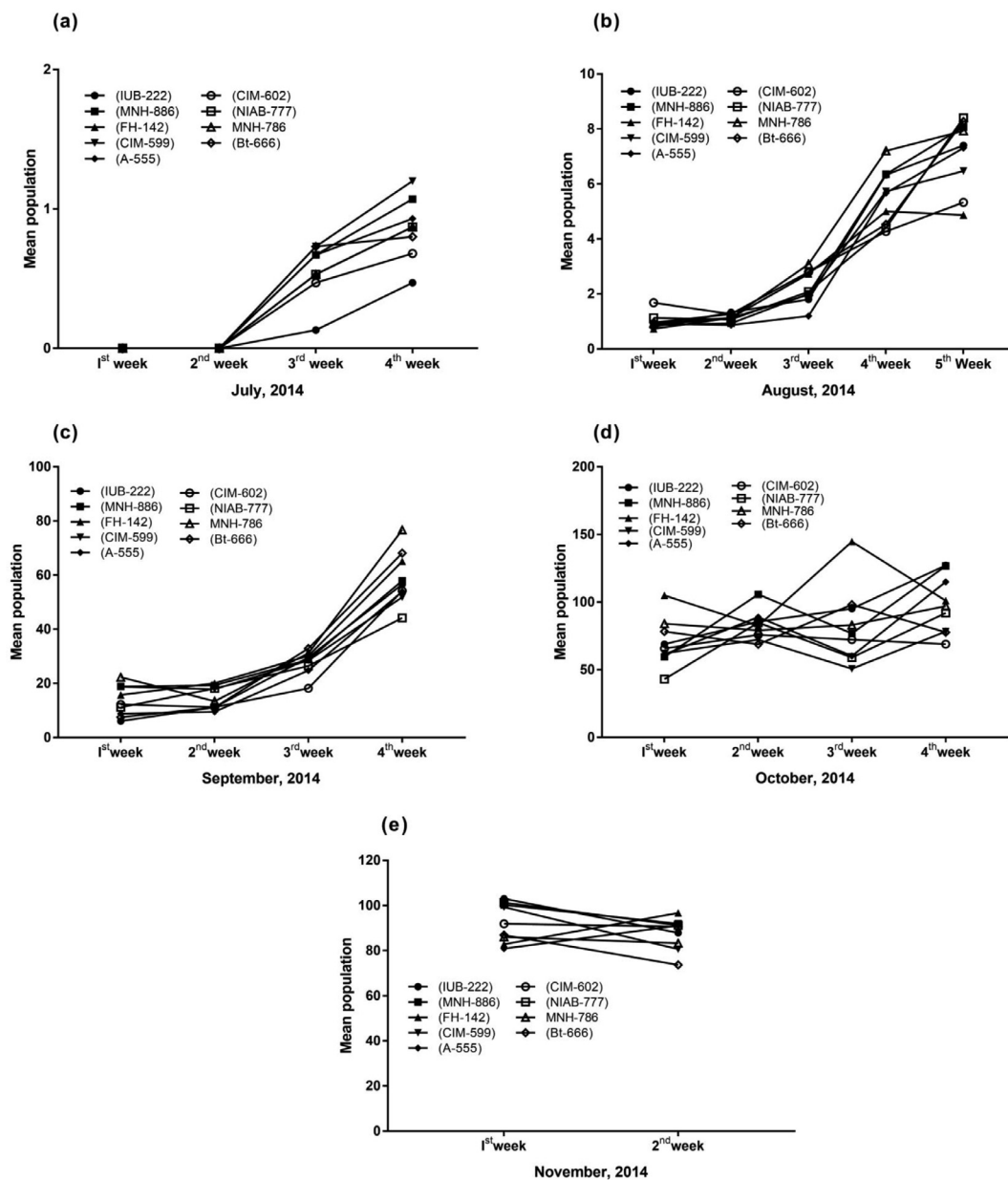


Fig. 1. Weekly population trend of DCB (a) July (b) August (c) September (d) October (e) November.

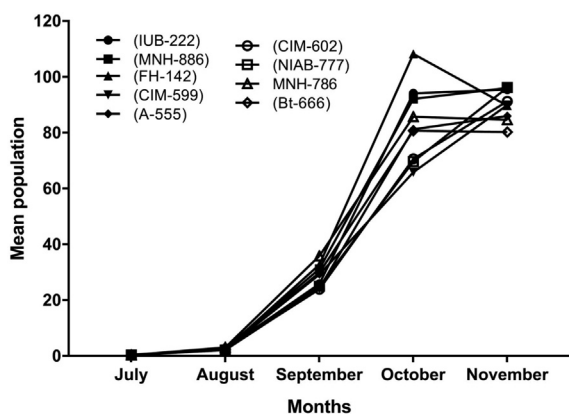


Fig. 2. Monthly trend of mean dusky cotton bug population during July–November.

like maximum lint discoloration, reduction in seed germination, seed weight and lint quality (Ahmed et al., 2015). These qualitative and quantitative losses are directly dependent upon bug density per plant (Khan et al., 2014). The overall mean by pooling DCB population on each variety for whole season revealed that three varieties (CIM-599, CIM-602 & IUB-222) showed a significantly lower population (37.76, 37.87, 43.84) and two varieties (FH-142, MNH-886) gave highest population (44.71, 46.81), respectively. In contrast, Shahid et al. (2017) reported that MNH-886 and FH-142 possessed minimum population of another stainer bug (red cotton bug) in comparison with transgenic varieties other than of our study.

All Bt cotton varieties bear differential DCB population and no variety was free from DCB which is confirmatory with DCB existence on different Bt varieties in different experimental setup (Men et al., 2003; Patil et al., 2007; Khan et al., 2014). The peak DCB population was observed during October which is in confirma-

Table 1

Seasonal occurrence of DCB during July–November, 2014.

Varieties	Mean population of DCB \pm SE					
	July	August	September	October	November	Overall mean
IUB-222	0.15 \pm 0.10 c	3.52 \pm 1.38 ab	25.87 \pm 11.42 cd	94.18 \pm 12.25 ab	95.47 \pm 7.53 ab	43.84 \pm 21.28 ab
MNH-886	0.43 \pm 0.26 ab	3.64 \pm 1.49 ab	31.22 \pm 9.19 ab	92.17 \pm 14.93 abc	96.10 \pm 4.17 a	44.71 \pm 20.88 a
FH-142	0.35 \pm 0.21 ab	2.88 \pm 0.90 c	32.77 \pm 11.19 ab	108.30 \pm 13.09 a	89.77 \pm 6.97 abcd	46.81 \pm 22.26 a
CIM-599	0.48 \pm 0.29 a	3.23 \pm 1.19 bc	29.25 \pm 7.91 bcd	65.88 \pm 6.07 d	89.97 \pm 9.37 abcd	37.76 \pm 17.57 c
A-555	0.40 \pm 0.24 ab	3.19 \pm 1.37 bc	24.27 \pm 10.55 d	81.33 \pm 13.03 bcd	86.00 \pm 5.07 bcd	39.04 \pm 18.70 bc
CIM-602	0.29 \pm 0.17 bc	3.08 \pm 0.77 bc	23.98 \pm 10.19 d	70.72 \pm 2.18 cd	91.30 \pm 1.43 abc	37.87 \pm 18.37 c
NIAB-777	0.35 \pm 0.21 ab	3.42 \pm 1.38 bc	25.00 \pm 7.12 cd	69.70 \pm 11.36 d	96.23 \pm 5.03 a	38.94 \pm 18.94 bc
MNH-786	0.35 \pm 0.21 ab	4.06 \pm 1.49 a	35.88 \pm 14.07 a	85.78 \pm 3.88 bcd	84.70 \pm 1.43 cd	42.15 \pm 18.64 abc
Bt-666	0.38 \pm 0.22 ab	3.56 \pm 1.34 ab	29.93 \pm 13.91 bc	80.75 \pm 6.20 bcd	80.30 \pm 6.57 d	38.98 \pm 17.72 bc
LSD at 0.05%	0.15	0.61	5.31	22.05	9.98	5.05

*Mean population/plant of three replicates sharing the common letters are not significantly different from each other. LSD: Least Significance Difference, SE: Standard Error, DCB: Dusky Cotton Bug.

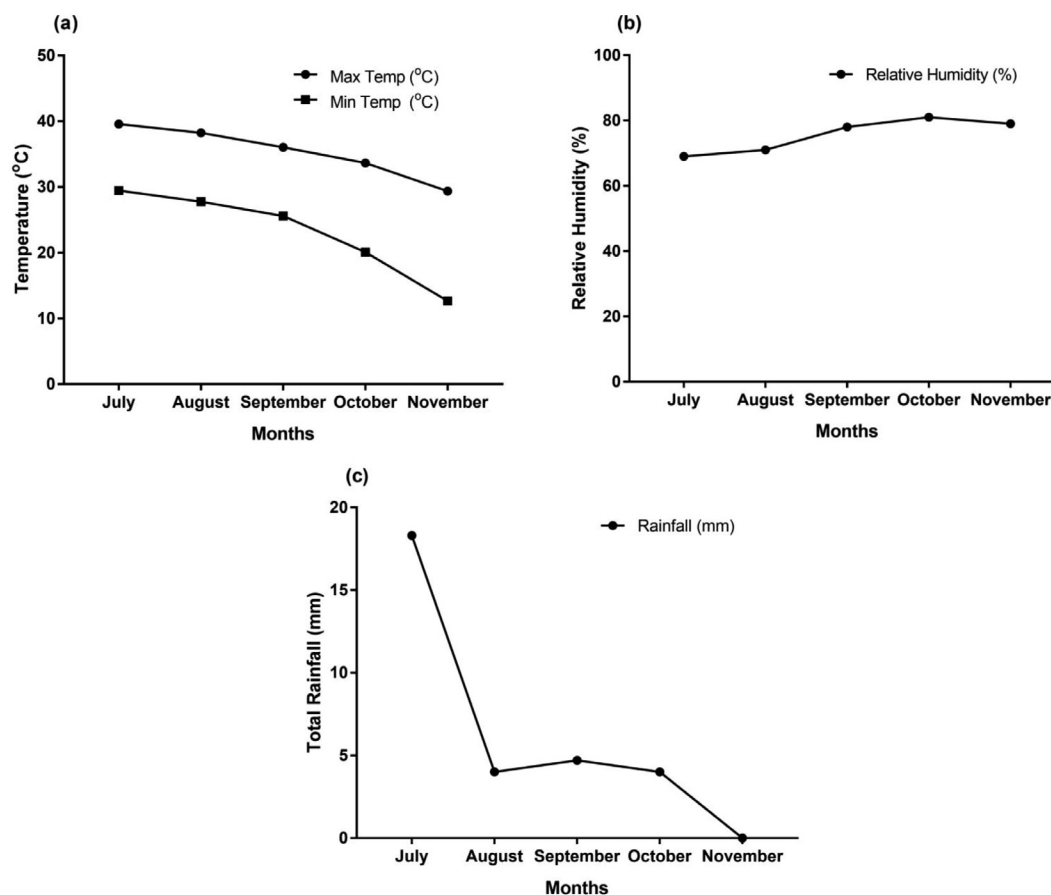


Fig. 3. Meteorological data during June–November, 2014 (a) Temperature (°C), (b) Relative humidity (%), (c) Rainfall (mm). Max: Maximum, Min: Minimum,

tion with previous study (Shahid et al., 2014). Red cotton bug (stainer bug) is also reported for its maximum population in September–October (Ashfaq et al., 2011) and October–November (Shah, 2014), and mealy bug in October–November (Hanchinal et al., 2010; Singh and Kumar, 2012). The reason of peak population might be the breeding of DCB that takes place on open bolls when the temperature is slightly lower during these months. The weather factors are also reported for their influencing effect on DCB population (Schaefer and Panizzi, 2000; Qayyoun et al., 2014). Thus, diversity in insects life cycle, required environmental parameters and host crop season could be the possible reasons for variation in peak population during different months.

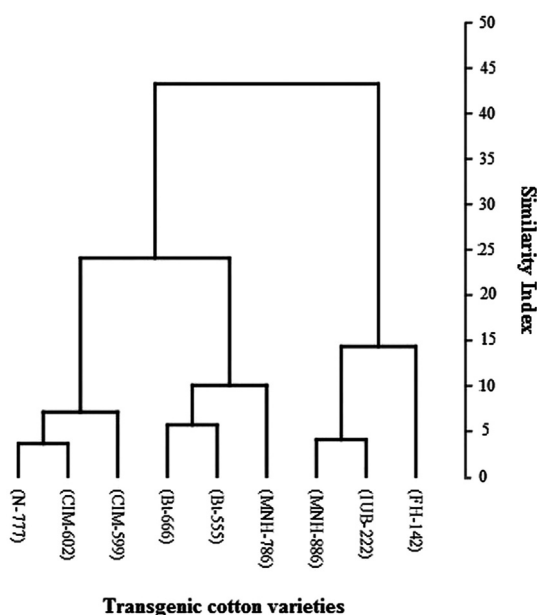
Fig. 3 exhibited the weather data regarding temperature, relative humidity and rainfall during the course of experiment.

Correlation matrix in table 2 indicated that DCB population exhibited significant negative correlation with temperature (maximum, minimum) and positive significant correlation with relative humidity which is in agreement with Hameed et al. (2014) but contradicts with the findings of Muthyala and Patil (2004) and (Srinivas and Patil, 2004b). Rainfall gave non-significant negative correlation with DCB population which is compatible with findings of Qayyoun et al. (2014). It is concluded that low temperature and high relative humidity favours the increase in DCB population. Thangavelu (1978) narrated that temperature and relative humidity are crucial for DCB infestation and growth. In cluster analysis with Ward's method- Euclidean distances, varieties were categorized into two main groups (Fig. 4). The first group consisted of FH-142, IUB-222 and MNH-886. The second group comprised of

Table 2

Correlation matrix of DCB with mean abiotic factors in transgenic cotton varieties.

Varieties	Abiotic factors		Relative humidity (%)	Rainfall (mm)
	Temperature (C°)			
	Max.	Min		
(IUB-222)	−0.920 [*]	−0.924 [*]	0.876 [*]	−0.628ns
(MNH-886)	−0.931 [*]	−0.930 [*]	0.894 [*]	−0.642ns
(FH-142)	−0.863ns	−0.860ns	0.908 [*]	−0.604ns
(CIM-599)	−0.981 ^{**}	−0.977 ^{**}	0.862ns	−0.674ns
(Bt-555)	−0.932 [*]	−0.935 [*]	0.877 [*]	−0.635ns
(CIM-602)	−0.970 ^{**}	−0.974 ^{**}	0.848ns	−0.657ns
(N-777)	−0.978 ^{**}	−0.982 ^{**}	0.836ns	−0.659ns
(MNH-786)	−0.919 [*]	−0.908 [*]	0.929 [*]	−0.655ns
(Bt-666)	−0.920 [*]	−0.915 [*]	0.912 [*]	−0.644ns

The asterisk indicates the significance level (* = Significant at $P \leq 0.05$, ** = highly significant $P \leq 0.01$, *** = highly significant $P \leq 0.001$, ns = non-significant).* Significant at $P \leq 0.05$,** highly significant $P \leq 0.01$.**Fig. 4.** Dendrogram (Ward's Method-Euclidean distances) showing the comparison of transgenic.

two sub groups (MNH-786, A-555, Bt-666) and (CIM-599, CIM-602, NIAB-777), respectively. It was interesting to know that IUB-222 possessed low population but two other varieties of same first group (FH-142, MNH-886) possessed very high DCB population. The clustering of FH-142 and MNH-886 in one group for DCB population is in agreement with findings of [Shahid et al. \(2017\)](#). CIM-599, CIM-602 with lowest mean DCB population were close to each and fall in second group.

4. Conclusion

The population of DCB was present in all the nine transgenic cotton varieties and no variety seemed to be resistant against DCB. However, the data revealed that there was comparatively less population of DCB recorded in CIM-599, CIM-602 and IUB-222, respectively. It is suggested that farmer may use these varieties with relatively low population dynamics to avoid the DCB population in the field. It will also help the farmer for understanding the attacking trend of DCB throughout the crop duration and to use the better management strategies at right time.

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