

EXPLORING BIOCHEMICAL BASES OF RESISTANCE TO FRUIT FLY INFESTATION IN SWEET GOURD CROPS

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ABSTRACT

Female fruit flies, *Bactrocera cucurbitae*, thrust eggs into developing cucurbitaceous fruits, and larval feeding inside the fruits leads to damage. The biochemical properties of fruits can act against this insect pest. This study was carried out with 12 sweet gourd germplasm lines (*Cucurbita moschata*), namely BD 264, BD 265, BD 266, BD 268, BD 269, BD 274, BD 275, BD 277, BARI Mistikumra 1, BARI Mistikumra 2, Gazipur local line, and China line, to assess fruit fly abundance and infestation on germplasm lines, and to find out the relationship between the infestation of fruit flies and the biochemical properties of sweet gourd fruit. The lowest abundance of fruit flies was observed on BD 277, followed by BD 265, BD 264, and BD 275. The lowest number of maggots was found in BD 274, followed by BD 264, and BD 277. In terms of fruit infestation, BD 274 and BD 277 were identified as resistant lines; BD 264, BD 265, BD 266, BD 268, BD 269, and BD 275 were classified as moderately resistant lines; and BARI Mistikumra 1, BARI Mistikumra 2, Gazipur local line, and China line were categorized as susceptible lines. The biochemical properties of the germplasm lines varied significantly. The infestation of fruit fly had significant positive correlation with the moisture content of BD 266, BARI Mistikumra 1 and BARI Mistikumra 2, and the reducing sugar content of BARI Mistikumra 1 and BARI Mistikumra 2.

Keywords: Abundance, *Bactrocera cucurbitae*, *Cucurbita moschata*, resistance

ABSTRAK

Betina lalat buah, *Bactrocera cucurbitae*, meletakkan telur pada buah cucurbitaceous yang sedang berkembang, dan peringkat larva memakan bahagian isi buah buah dan menyebabkan kerosakan. Kandungan biokimia buah dapat bertindak melawan serangga perosak ini. Kajian ini dilakukan dengan 12 jalur germplasma labu manis (*Cucurbita moschata*), iaitu BD 264, BD 265, BD 266, BD 268, BD 269, BD 274, BD 275, BD 277, BARI Mistikumra 1, BARI

Mistikumra 2, Gazipur garis tempatan, dan garis China, untuk menilai kelimpahan dan infestasi lalat buah pada saluran germplasma, dan untuk mengetahui hubungan antara serangan lalat buah dan kandungan biokimia buah labu manis. Kelimpahan lalat buah paling rendah diperhatikan pada BD 277, diikuti oleh BD 265, BD 264, dan BD 275. Bilangan berenga terendah dijumpai di BD 274, diikuti oleh BD 264, dan BD 277. Dari segi serangan buah, BD 274 dan BD 277 dikenal pasti sebagai garis rentan; BD 264, BD 265, BD 266, BD 268, BD 269, dan BD 275 dikelaskan sebagai garis rentan sederhana; dan BARI Mistikumra 1, BARI Mistikumra 2, garis tempatan Gazipur, dan garis China dikategorikan sebagai garis tidak rentan. Kandungan biokimia dari garis germplasma bervariasi dengan ketara. Kelimpahan lalat buah mempunyai korelasi positif yang signifikan dengan kandungan kelembapan BD 266, BARI Mistikumra 1 dan BARI Mistikumra 2, dan penurunan kandungan gula BARI Mistikumra 1 dan BARI Mistikumra 2.

Kata kunci: Kelimpahan, *Bactrocera cucurbitae*, *Cucurbita moschata*, rentan

INTRODUCTION

The sweet gourd, *Cucurbita moschata*, is a popular vegetable all over the world (Dhaliwal 2017); hence, it is a crop of utmost economic importance. It is distinguishable because of its long shelf life, high nutritive value, and low cost, but cucurbit fruit fly *Bactrocera cucurbitae* (Diptera: Tephritidae) infestation causes direct economic loss to it (Wee & Hee 2018). The female insects damage the fruit by inserting their eggs beneath the skin of the fruits, which later on develop into larvae and feed on the pulp of the fruits. Amin et al. (2011) studied the fruit infestation by fruit flies using different cucurbits and found that sweet gourds face the highest infestation rate (71.5%), whereas ridge gourd faced the lowest infestation (21.0%). They recorded sweet gourd as the most preferred host for fruit fly infestation considering its short pre-mating, pre-oviposition, incubation, and larval periods on sweet gourd. Sapkota et al. (2010) found 54.3% fruit damage in squash due to fruit fly infestation in untreated control condition.

Fruit flies frequently attack sweet gourd, bitter gourd, snake gourd, bottle gourd, ridge gourd, cucumber, melon, mango, papaya, banana, apple, etc. (Dhillon et al. 2005; Gazmer et al. 2017; Rattanapun et al. 2009). Female fruit flies use their olfactory, visual, and tactile cues to find and assess hosts for egg deposition and larval development (Brevault & Quilici 2007). They attack all stages of fruits, but they prefer ripe and fully ripe fruits over unripe fruits as these are best for larval survival, a high percentage of pupation, and short developmental periods. It is also reported that the organic volatile compounds released from ripe fruits attract fruit flies (Rattanapun et al. 2009). The infested fruits become damaged by the destruction of the inner tissues of fruits through larval feeding, and enhancing the attack of other pests and microorganisms through oviposition punctures (Clarke et al. 2005).

Plants possess different mechanisms of resistance which enable plants to avoid, minimize, or tolerate the effects of pest attacks (Sarfraz et al. 2006). The changes in total soluble solids in mangoes from being unripe until ripening influence the fruit flies' preference for selecting oviposition sites (Rattanapun et al. 2009). According to a report by Gazmer et al. (2017), the amount of total sugar and reducing sugar was lowest in resistant varieties, whereas it was highest in susceptible varieties of sweet gourd. They also found that the total sugar, reducing sugar, non-reducing sugar, and pH of fruit had significant positive correlations with the percentage of fruit infestation and larval density per fruit of sweet gourd.

Plant resistance is a complex phenomenon, and several factors are responsible for this. The biochemical properties of plants can repel insect attacks (Haldhar et al. 2013). Recognizing these biochemical factors can be helpful in identifying resistant lines for variety development. This type of resistance is also helpful in minimizing insecticide application while causing no environmental harm.

Conventional insecticide application cannot effectively control this pest, and its excessive use results in the development of insect resistance, secondary pest outbreak, hazards to non-target organisms, and imbalances in the ecosystem. Therefore, eco-friendly management of fruit flies is a prime need for commercial sweet gourd production. Considering this fact, the current study was carried out to explore the differential levels of fruit fly infestation on twelve sweet gourd germplasm lines and to assess the contribution of the biochemical properties of the tested germplasm lines to resist fruit fly attack.

MATERIALS AND METHODS

Study Site and Conditions

Our study was conducted in the field and laboratory of the Department of Entomology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh from July 2018 to June 2019. The study site is located at 25°25' N and 89°5' E and surrounded by a sal tree (*Shorea robusta*) forest. The annual mean maximum temperature, annual mean minimum temperature, relative humidity, and rainfall are 36.0°C, 12.7°C, 65.8%, and 2376 mm, respectively (Amin et al. 2019).

Cultivation of the Sweet Gourd Germplasm Lines

The tested sweet gourd germplasm lines were BARI Mistikumra 1, BARI Mistikumra 2, BD 264, BD 265, BD 266, BD 268, BD 269, BD 274, BD 275, BD 277, Gazipur local line, and China line. The germplasm seeds were collected from Bangladesh Agricultural Research Institute, Gazipur and Department of Entomology, BSMRAU.

The germplasm lines were cultivated in the field following a randomized complete block design with plot size of 4.0 m × 3.0 m, which were carried out with three replications. There were 36 plots in total, and each of them contained three pits. A distance of 1.0 m was maintained in each case of block-to-block and plot-to-plot spacing. First, the seedlings of each germplasm were raised in polybags, and then the 3-week-old seedlings were transplanted to the field on December 4th, 2018. All intercultural operations, except insect control measures, were undertaken based on necessity. Fertilizers were applied according to the Fertilizer Recommendation Guide (FRG 2018) (N, 120 kg; P, 70 kg; K, 40 kg; and S, 20 kg per hectare).

Observation of Cucurbit Fruit Fly

Data were collected from five randomly selected plants per germplasm. Fruit flies started to appear in the field from the flowering stage. Hence, the number of adult fruit flies per plant was counted starting from 56 days after transplanting, which was also marked as the start of the crop's reproductive stage, and continued until the final harvest. Visual observation was done to count the number of adult fruit flies irrespective of male and female insects. Therefore, the visitation of adult fruit fly was observed per plant basis for fifteen minutes from 8.00 am to 11.00 am. Data were collected maintaining weekly intervals. Three infested fruits from each of five selected plants of all germplasm lines were collected, brought to the laboratory, and cut into pieces for counting the number of maggots per fruit. The total number of fruits and the number of infested fruits of the selected plants were counted at weekly intervals till the final

harvest to calculate the fruit infestation percentage. Moreover, based on the days after formation, the infested fruits were separated as early, premature and mature infested fruits (<3weeks, early stage; 3-6weeks, prematurity age; and >6 weeks, maturity stage).

Estimation of Fruit Biochemical Properties

Fresh matured fruits from each germplasm were used for biochemical analysis. The moisture content of the fruits was determined through oven-drying method. A spectrophotometric method was used to determine the beta-carotene content of the fruit samples following the procedure previously explained by Azeez et al. (2012). Reducing sugar and total sugar content of the fruit of each germplasm were estimated using Bertrand's method (Kumar et al. 2011). The nitrogen content of the fruits was estimated using Micro Kjeldahl method (Maehre et al. 2018). Each of the values of nitrogen content was then multiplied by 6.25 to get the percentage of protein.

Categorization of the Germplasm Lines Based on Fruit Fly Infestation Level

The tested germplasm lines were graded into different categories of resistance according to the susceptibility rating scale previously described by Gazmer et al. (2017). The rating scale for resistance categorization includes immune, highly resistant, resistant, moderately resistant, susceptible, and highly susceptible; with the corresponding fruit infestation percentages of 0%, 1–10%, 11–20%, 21–50%, 51–75%, and >76%, respectively.

Data Analysis

A one-way analysis of variance, followed by Tukey's honestly significant difference (HSD) post hoc test at 5% level of significance, was used to determine the variations of mean abundance of adult fruit fly and maggots on the tested germplasm lines, and to compare the means of biochemical properties of the germplasm lines. Pearson's correlation coefficients were used to determine the relationship between the biochemical properties of fruits and the fruit fly infestation level of the germplasm lines. All analyses were performed using the IBM SPSS 21.0 software (IBM SPSS Statistics, Armonk, NY, USA).

RESULTS

The mean number of adult fruit fly among the germplasm lines varied from 1.4 ± 0.2 to 2.5 ± 0.2 plant⁻¹, and the results significantly differed ($F_{11, 528} = 6.2$, $P < 0.001$). The lowest population of fruit flies was observed in BD 277, followed by BD 265, BD 264, BD 275, BD 274, BD 268, BD 266, and BD 269 (1.4 ± 0.2 , 1.4 ± 0.1 , 1.5 ± 0.1 , 1.5 ± 0.2 , 1.6 ± 0.2 , 1.6 ± 0.1 , 1.8 ± 0.2 and 1.8 ± 0.2 per plant, respectively) (Figure 1).

The number of maggots per fruit significantly varied among the twelve sweet gourd germplasm lines ($F_{11, 48} = 3.6$, $P < 0.05$), ranging from 30.4 ± 8.9 to 107.0 ± 8.6 . The lowest number of maggots was found in the infested fruits of BD 274, followed by BD 264, BD 277, BD 268, BD 269, BD 275, BD 265, and BD 266 (30.4 ± 8.9 , 32 ± 5.1 , 33 ± 7.3 , 52 ± 14.6 , 55.4 ± 21.6 , 59 ± 18.5 , 69.4 ± 11.5 and 87.4 ± 8.3 per fruit, respectively) (Figure 2).

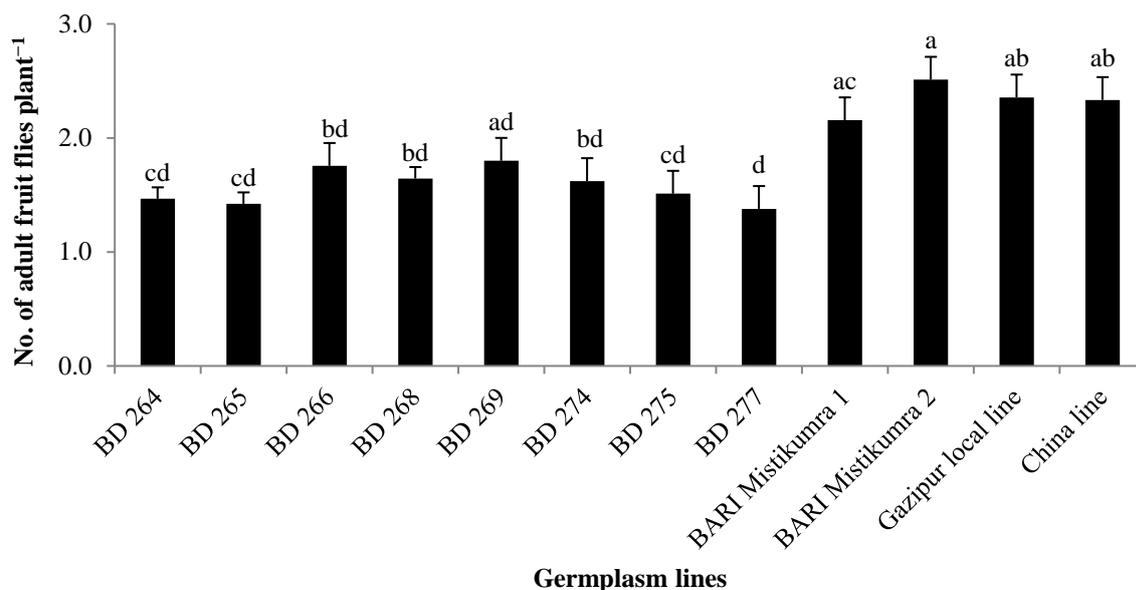


Figure 1. Abundance of adult fruit flies (mean±SE) on 12 sweet gourd germplasm lines from December 2018 to March 2019

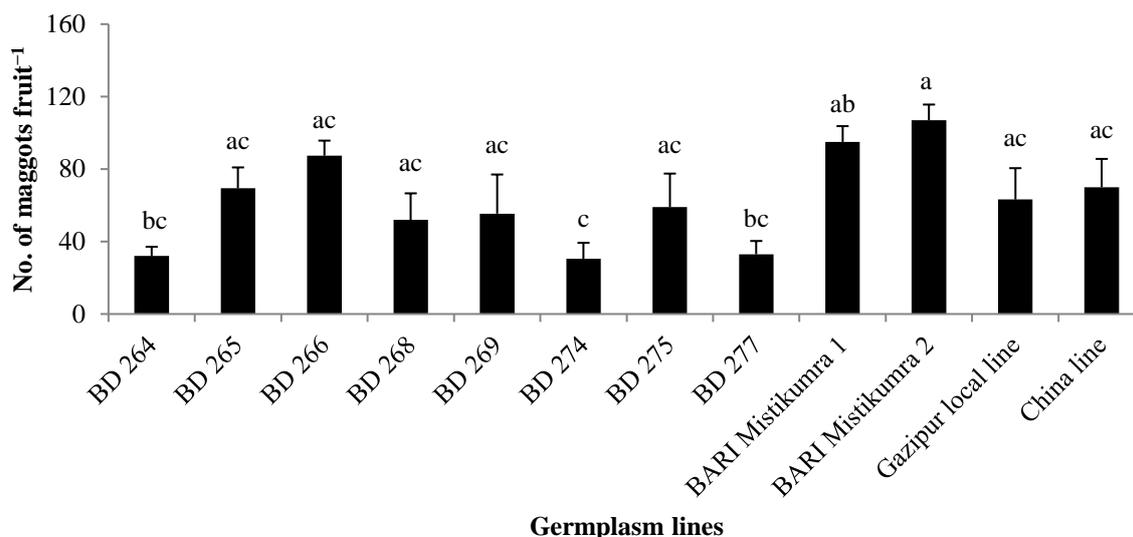


Figure 2. Number of fruit fly maggots per fruit (mean±SE) in 12 sweet gourd germplasm lines from December 2018 to March 2019

Considering the percentage of fruit infestation, the germplasm lines were classified following the susceptible rating scale (Table 1). Relatively lower fruit infestation levels were recorded in BD 277 (19.3%) and BD 274 (19.7%). They were classified as resistant, because their fruit infestation rates ranged from 11% to 20%. The fruit infestation rates of BD 275, BD 266, BD 269, BD 264, BD 268, and BD 265 were 33.2%, 37.0%, 37.1%, 37.2%, 37.8%, and 43.2%, respectively, which were within the range of 21–50%. Hence, they were categorized as moderately resistant. BARI Mistikumra 1, Gazipur local line, China line, and BARI Mistikumra 2 showed fruit infestation rates 51.6%, 53.5%, 55.0%, and 57.0%, respectively. The rates of fruit infestation of these germplasm lines were within the range of 51–75%; hence, they were

grouped as susceptible. The rate of fruit infestation by fruit fly tended to increase with the increase of fruit age. The maximum rate of fruit infestation was observed at maturity stage of fruits in all the germplasm lines.

Table 1. Categorization of germplasm lines based on percentage of fruit infestation by fruit flies

Germplasm lines	Fruit infestation (%)			Total	Rating
	At early stage	At prematurity stage	At maturity stage		
BD 264	8.5	11.4	17.3	37.2	Moderately resistant
BD 265	9.4	13.3	20.5	43.2	Moderately resistant
BD 266	8.3	11.8	19.4	37.0	Moderately resistant
BD 268	7.5	12.0	18.3	37.8	Moderately resistant
BD 269	7.8	12.6	17.7	37.1	Moderately resistant
BD 274	4.0	6.3	9.4	19.7	Resistant
BD 275	5.4	12.2	16.6	33.2	Moderately resistant
BD 277	2.7	6.5	10.1	19.3	Resistant
BARI Mistikumra 1	12.1	12.7	26.8	51.6	Susceptible
BARI Mistikumra 2	13.0	13.3	30.7	57.0	Susceptible
Gazipur local line	12.8	13.8	26.9	53.5	Susceptible
China line	12.3	15.6	27.1	55.0	Susceptible

The tested germplasm lines significantly varied in terms of fruit biochemical properties (moisture: $F_{11,24}=8.2$, $P<0.001$; beta-carotene: $F_{11,24}=412.7$, $P<0.001$; reducing sugar: $F_{11,24}=9.0$, $P<0.001$; total sugar: $F_{11,24}=13.4$, $P<0.001$; and protein: $F_{11,24}=7.6$, $P<0.001$). The fruit moisture content was lowest in BD 274 and BD 277 (85.1% and 85.8%, respectively), followed by BD 275, BD 268, China line, BD 265, and BD 269 (Table 2). The amount of beta-carotene was the lowest in BD 269 (1.6 ± 0.1 mg/100g fresh wt.), followed by BD 268, BD 274, BD 265, BD 266, BD 264, and BD 277. The lowest content of reducing sugar was found in BD 277 (5.2%), followed by BD 268, BD 264, and BD 275. The total sugar content was the lowest in BD 274, BD 268, and BD 277 (6.8%, 6.9% and 7.0%, respectively), followed by BD 269 and BD 275. The protein content in the fruits was the lowest in BD 277 and BD 266 (3.8% and 3.9%, respectively), followed by BD 274 and BD 264 (Table 2).

Table 2. Comparisons of biochemical properties (mean±SE) of fruits among the tested germplasm lines

Germplasm lines	Moisture (%)	Beta-carotene (mg/100g fresh wt.)	Reducing sugar (%)	Total sugar (%)	Protein (%)
BD 264	93.1±0.1ab	3.6±0.3f	5.6±0.2cd	7.4±0.3ce	4.1±0.2bc
BD 265	89.6±1.0bc	2.7±0.1fg	6.4±0.3bc	7.7±0.4be	5.0±0.2ab
BD 266	95.7±0.2a	2.8±0.1f	6.5±0.4ac	7.4±0.4ce	3.9±0.2c
BD 268	88.7 ± 1.2 bc	2.2±0.0gh	5.4±0.3cd	6.9±0.2e	4.4±0.1ac
BD 269	89.7±1.2bc	1.6±0.1h	5.9±0.1bd	7.1±0.3de	5.0±0.2ab
BD 274	85.1±2.1c	2.6±0.0fg	6.2±0.2bd	6.8±0.2e	4.1±0.2bc
BD 275	88.3 ± 0.8 bc	7.4±0.1b	5.6±0.1cd	7.1±0.1de	5.3±0.2a
BD 277	85.8 ± 1.4 c	4.0±0.0f	5.2±0.2d	7.0±0.2e	3.8±0.3c
BARI Mistikumra 1	91.2±0.7ab	4.8±0.0cd	6.0±0.2bd	8.6±0.2ac	5.0±0.1ab
BARI Mistikumra 2	90.3±0.9ac	10.3±0.0a	6.3±0.3bd	8.9±0.3ab	4.9±0.1ab
Gazipur local line	92.8±0.8ab	5.3±0.3c	6.9±0.3ab	8.3±0.2bd	4.4±0.2ac
China line	89.5±0.5bc	4.7 ± 0.1 e	7.6±0.2a	9.7±0.3a	5.3±0.3a

Means within a column followed by same letter(s) are not significantly different according to Tukey's HSD post hoc test at <0.05 significance level.

Fruit fly infestation on all the tested germplasm lines was positively correlated with all the estimated biochemical properties of fruits, that is, moisture, beta-carotene, reducing sugar, total sugar, and protein contents (Table 3). Among the germplasm lines, BD 266, BARI Mistikumra 1, and BARI Mistikumra 2 showed a statistically significant positive correlation between moisture content and fruit fly infestation. Moreover, fruit fly infestation on BARI Mistikumra 1 and BARI Mistikumra 2 showed a statistically significant positive correlation with reducing sugar content of the fruits. All other results were statistically insignificant.

Table 3. Correlation coefficients between biochemical properties (mean±SE) of fruits and fruit fly infestation on the tested germplasm lines

Germplasm lines	Moisture (%)	Beta-carotene (mg/ 100g fresh wt.)	Reducing sugar (%)	Total sugar (%)	Protein (%)
BD 264	0.791 ^{NS}	0.715 ^{NS}	0.320 ^{NS}	0.391 ^{NS}	0.703 ^{NS}
BD 265	0.673 ^{NS}	0.660 ^{NS}	0.611 ^{NS}	0.493 ^{NS}	0.886 ^{NS}
BD 266	0.998*	0.213 ^{NS}	0.560 ^{NS}	0.599 ^{NS}	0.854 ^{NS}
BD 268	0.930 ^{NS}	0.433 ^{NS}	0.927 ^{NS}	0.777 ^{NS}	0.967 ^{NS}
BD 269	0.286 ^{NS}	0.958 ^{NS}	0.784 ^{NS}	0.944 ^{NS}	0.555 ^{NS}
BD 274	0.396 ^{NS}	0.525 ^{NS}	0.950 ^{NS}	0.332 ^{NS}	0.500 ^{NS}

BD 275	0.989 ^{NS}	0.977 ^{NS}	0.964 ^{NS}	0.893 ^{NS}	0.215 ^{NS}
BD 277	0.698 ^{NS}	0.875 ^{NS}	0.733 ^{NS}	0.778 ^{NS}	0.959 ^{NS}
BARI	0.998*	0.833 ^{NS}	0.997*	0.947 ^{NS}	0.962 ^{NS}
Mistikumra 1					
BARI	0.997*	0.822 ^{NS}	0.998*	0.996 ^{NS}	0.971 ^{NS}
Mistikumra 2					
Gazipur local line	0.972 ^{NS}	0.823 ^{NS}	0.932 ^{NS}	0.911 ^{NS}	0.988 ^{NS}
China line	0.979 ^{NS}	0.927 ^{NS}	0.994 ^{NS}	0.981 ^{NS}	0.833 ^{NS}

NS, Non-significant ($p \geq 0.05$)

*Significant ($p < 0.05$).

DISCUSSION

Various management strategies are practiced to keep fruit fly (*B. cucurbitae*) infestation below economically injurious level to stabilize sweet gourd production worldwide. Among such strategies, selecting resistant genotypes is the most effective method to minimize the damage of this insect on sweet gourd fruits to obtain favorable qualitative and quantitative yield. This study investigated the role of different sweet gourd genotypes in resisting *B. cucurbitae* infestation, highlighting the relationship between the fruit biochemical contents of the genotypes and level of fruit fly infestation.

The present study found a range of 1.4 to 2.5 adult fruit flies per plant and the number of maggots varied from 30.4 to 107.0 per fruit depending on the tested sweet gourd germplasm lines. In the case of fruit fly larval density, Haldhar et al. (2015) found 13.17 to 28.43 larvae per ridge gourd fruit. They added that larval density per fruit was significantly lower in resistant genotypes. Dhillon et al. (2005) reported 3.4 to 7.8 fruit fly larvae per fruit of bitter gourd. It is clear that the population of adult flies and larvae varies based on the host plant.

The results of this study revealed different fruit fly infestation levels on the tested sweet gourd germplasm lines. The fruit infestation levels of the germplasm lines were within the range of 19.3–57.0%. Duradundi et al. (2018) reported that fruit flies caused 9.91–78.27% fruit infestation on different genotypes of ridge gourd. This variation may have happened due to different host plants, climatic conditions, cropping seasons, and geographical locations.

The biochemical properties of sweet gourd also varied among its germplasm lines. Fedha (2008) found average moisture content of 87.9% in the fruits of *Cucurbita moschata*. Sharma and Rao (2013) reported that carotenoids were found to be the highest in ripened fruits of sweet gourd (7.47 mg/100 g Fresh weight). The variations of these properties affected adult fruit flies in selecting the oviposition site. Nath et al. (2017) stated that the tested germplasm lines of bitter gourd showed significant differences in fruit infestation and larval density per fruit. They also found that the moisture content of the fruits have a significant positive effect on fruit damage. Haldhar et al. (2013) found that total sugar, reducing sugar, non-reducing sugar, and pH were consistently low in resistant muskmelon genotypes against fruit fly infestation but were high in susceptible genotypes. Dhillon et al. (2005) observed that the moisture content of bitter gourd had a positive and significant association with fruit fly infestation and larval density.

Ingoley et al. (2005) found that susceptible and highly susceptible genotypes of cucumber to fruit fly infestation contained higher amounts of total sugars but lower amount of

phenols, compared with moderately resistant genotypes. In this study, BD 277 and BD 274 contained relatively low amount of moisture, total sugar, and protein. This may be the reason why these germplasm lines showed resistance to fruit fly infestation.

Host plant selection by insects is highly dependent on their nutritional requirements, safeguard for oviposition, and complete larval development (Gogi et al. 2010). The volatile lure of cucurbits had a strong female-biased attraction to fruit flies (Royer et al. 2014), and *B. cucurbitae* flies have good chemical perception (Dhillon et al. 2005). Thus, the presence of some chemicals, such as moisture, reducing sugar, non-reducing sugar, nitrogen, protein, flavonoid, tannins, and phenols in fruits may serve as attractant or deterrent to fruit flies (Dhillon et al. 2005; Haldhar et al. 2018). Hence, the fruit flies' perception of fruit biochemical properties influences its oviposition preference and multiplication, that is, the number of larvae per fruit.

As the maggots remain inside the fruit, the application of insecticides cannot effectively control them. Moreover, insecticide application entails many hazards. Hence, identifying the resistant plant genotypes can be a good solution for this predicament. The findings of this study clearly showed that fruit fly infestation had a positive correlation with the estimated biochemical properties of sweet gourd. Since no insect control measure was applied in the entire study, the germplasm lines that were less affected by fruit fly infestation resisted pest attacks by themselves. These biochemical properties of sweet gourd, which demonstrated resistance against fruit fly infestation, can be used in plant breeding programs as marker traits to select resistant genotypes. Hybridization techniques may be employed to transfer resistant genes from resistant genotypes in the cultivated genotypes of sweet gourd to develop resistant varieties. In conclusion, the cultivation of infestation-resistant and -moderately resistant varieties of sweet gourd should be emphasized to minimize the infestation of fruit flies in sweet gourd crop production.

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