

# **BIO-PHYSICAL BASES OF ANTIXENOTIC MECHANISM OF RESISTANCE IN BITTER-GOURD (*MOMORDICA CHARANTIA* L., CUCURBITACEAE) AGAINST MELON FRUIT FLY, *BACTROCERA CUCURBITAE* (COQUILLET) (DIPTERA: TEPHRITIDAE)**

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## **Abstract**

Plants genotypes possess different phenotypic and/or biochemical properties, which resultantly induce in them different mechanisms of resistance. These mechanisms enable the plants to avoid, tolerate or recover from the effects of insect pest attacks. The results of the present studies revealed that there were significant variation in tested bitter-gourd genotypes for percentage fruit-infestation and larval-density per fruit. Col-II and Faisalabad-Long were ranked resistant genotypes and identified as resistance source for melon fruit fly, *Bactrocera cucurbitae*. The larval density per fruit had a significant positive correlation ( $r = 0.992$ ) with percentage fruit infestation. The fruit-length, fruit-diameter, number of longitudinal ribs/fruit and number of small ridges/cm<sup>2</sup>, which were significantly lowest in resistant and highest in susceptible genotypes, had a significant positive correlation with the percent fruit infestation and larval-density per fruit. However, fruit toughness, height of small ridges, height of longitudinal ribs and pericarp thickness, which were significantly highest in resistant and lowest in susceptible genotypes, had a significant negative correlation with the percent fruit infestation and larval-density per fruit. Step-wise multiple regression analysis indicated that the tested morphological traits explained 100% of the total variation in fruit infestation and larval-density per fruit. However, the fruit-length, fruit-diameter, fruit-toughness and number of longitudinal ribs showed 95.49% of the total variation in fruit fly infestation and 99.67% of the total variation in the larval-density per fruit. The maximum variation, in fruit infestation and larval-density per fruit, was explained by fruit toughness (63.4 and 49.2%, respectively) followed by fruit-diameter (23.22 and 22.34%, respectively) and number of longitudinal ribs (8.23 and 11.57%, respectively). These can be used as marker traits to induce resistance against melon fruit fly in bitter gourd; whereas, rest of the morphological fruit-traits explained less than 2% variation in the fruit infestation and less than 1% variation in the larval-density per fruit.

## **Introduction**

In nature, plant genotypes are exposed to various types of stressors, like those of the nutrients imbalance as well as of the soil composition (Scriber, 1984a; Eckey-Kaltenbach *et al.*, 1994; Goncalves-Alvim *et al.*, 2004), micro-climate, plant-genetics, plant-tissue ontogeny (Ponti, 1977; Scriber, 1984b; Mutikainen *et al.*, 2000), herbivore (or abiotic) induction-responses (Tallamy & Raupp, 1991), somatic-mutations (Karban & Baldwin, 1997), plant-chemistry (Feeny, 1995; Mutikainen *et al.*, 2000; Masood *et al.*, 2005) and/or of the interplay between all of them (Stadler, 1992). These stressors alter not only the genotypic, but also the phenotypic and biochemical properties of the plants and resultantly, induce in them different mechanisms of resistance, which enable them to avoid, tolerate or recover from the effects of insect pest attacks (Tingey, 1986; Eckey-Kaltenbach *et al.*, 1994; Pedigo, 1996; Shaheen *et al.*, 2006). These mechanisms of

resistance mostly induce the morphological and biochemical features of plant (Carter, 1927; Feeny, 1995; Stadler, 2000; Hirota & Kato, 2001; Goncalves-Alvim *et al.*, 2004; Afzal *et al.*, 2009) that impair the normal feeding or oviposition of various pest-insects (Harrison & Karban, 1986; Morris & Dwyer, 1997; Underwood, 1999; Shaheen *et al.*, 2006; Afzal & Bashir, 2007; Afzal *et al.*, 2009) or induce other mortality factors, that collectively create phonetic resistance (Kogan, 1982; Coley, 1983; Coley & Barone, 1996). These mechanisms of resistance have proved to be effective tools against the pest-insects in many crops and vegetables (Eigenbrode & Trumble, 1994; Felkl *et al.*, 2005).

The mechanisms of resistance in plants are either constitutive or induced (Painter, 1951; Karban *et al.*, 1997; Karban & Agrawal, 2002; Traw & Dawson, 2002) and are grouped into three main categories, viz., non-preference or antixenosis, antibiosis and tolerance (Painter, 1951).

Antixenotic mechanism of resistance, which is employed by the host plants, deters the insects from oviposition (Painter, 1951; Valencia, 1984; Karban, *et al.*, 1997; Afzal *et al.*, 2009), feeding, seeking shelter (Dabrowski & Kidiavai, 1983; Woodhead & Taneja, 1987; Sharma & Nwanze, 1997) and colonization (Dhaliwal & Arora, 2003). This mechanism renders the plants undesirable or, in other words, to be bad hosts for rather an easy invasion of insects (Bazzaz *et al.*, 1987; Schoonhoven *et al.*, 1998; Dhaliwal & Arora, 2003). Antixenosis, exhibited by the plants may be due to certain morphological characteristics of different plant parts, *vis-à-vis* certain allelochemicals present in them (Kogan, 1982; Rhoades, 1983; Edelman-Keshet, 1986; Edelman-Keshet & Rausher, 1989; Adler & Karban, 1994; Morris & Dwyer, 1997; Thaler, 1999; Afzal *et al.*, 2009) or various interactions among these factors-groups (Panda & Khush, 1995), that resultantly induce one or more breaks in the chain of responses leading towards oviposition or feeding (Panda & Khush, 1995; Dhaliwal & Arora, 2003).

There are significant differences in genotypic susceptibility to melon fruit fly among bitter-gourd cultivars (Dhillon *et al.*, 2005) which suggest the need to identify sources of resistance to the target pests, followed by an identification of physio-chemical factors involved in host plant selection by the insects either for oviposition or feeding (Painter, 1951; Maxwell & Jennings, 1980) and larval performance (Fitt, 1986; Hendrichs *et al.*, 1995). Hence, the development of varieties resistant to melon fruit fly is an important component of an integrated pest management program for melon fruit fly (Panda & Khush, 1995). The development and then the cultivation of fruit fly-resistant bitter-gourd cultivars has been impaired, because of the lack of adequate information on the sources of plant and fruit-traits associated with resistance and their influence on the pest-multiplication (Dhillon *et al.*, 2005). Therefore, it becomes imperative to identify physical and biochemical fruit-traits associated with resistance (Dhillon *et al.*, 2005) and get knowledge of their influence on oviposition preference, larval performance (Fitt, 1986) and pest multiplication (Dhillon *et al.*, 2005) for devising sustainable pest management strategies for the control of fruit flies (Fitt, 1986). The bitter-gourd cultivars and/or genotypes resistant to the melon fruit flies on the basis of biophysical and biochemical fruit-traits have not yet been identified. This study was, therefore, planned to screen out resistant genotypes of the available bitter-gourd accessions in Pakistan, in order to determine their biophysical sources of resistance against the melon fruit fly.

## Materials and Methods

**Preliminary screening of varieties:** Thirteen varieties of bitter-gourd viz., Col-II, FSD-long, Col-Nankana sahib, Col-I, GS-51, Col-III, Col-Multan, Col-Vehari, Chaman,

Sunder-F1, Janpuri, F1-484 and F1-485, were sown at two localities, i.e. Ayub Agricultural Research Institute, Faisalabad and Chak No. 103-04/7R, Harappa, Sahiwal. The seeds of each variety were soaked in water in petridishes for two hours, before sowing, to soften the seed-coat. The sowing was done on 10 April, 2005, at Faisalabad and on 15 April, 2005, at Harappa. The experiment was laid out in a Randomized Complete Block Design, with three replications of each variety. The area of each block (bed) was 6m X 2m. In each experimental unit, the plant to plant distance was maintained at 30 cm. All the recommended agronomic practices were carried out. But none of the fruit fly management practices were carried out in order to check the varieties' resistance of tested bitter-gourd varieties against the melon fruit fly. Picking of the fruits was started on 10 June, 2005, at Faisalabad and on 15 June, 2005, at Harappa. Totally, five pickings were done at each locality. After each picking, the fruits were weighed with a weighing balance, in the field. After weighing, ten fruit were randomly taken from each replicate of each genotype and were brought into the laboratory, where they were observed for fruit-infestation, under a microscope. The infested fruits were counted and the percent fruit-infestation was calculated. Each infested fruit was then observed under a microscope, the number of larvae per fruit were counted. The genotypes were grouped by following the rating system, given by Nath (1966) for the fruit damage as immune (no damage), highly resistant (1–10%), resistant (11–20%), moderately resistant (21–50%), susceptible (51–75%) and highly susceptible (76–100%).

**Rescreening of the selected varieties:** Promising six varieties of bitter-gourd, viz., two susceptible (Chaman and Vehari), two moderately resistant (Col-1 and Nankana Sahib) and two resistance ( Col-2 and Faisalabad-long) were sown during 2006 at two localities, i.e., Ayub Agricultural Research Institute, Faisalabad and Chak No. 103-04/7R, Harappa, Sahiwal in the same way as described earlier. After each picking, the fruits were separated into marketable (uninfested) and nonmarketable (infested) fruits and weighed with a weighing balance, in the field. The infested fruits were counted and the percent fruit-infestation was calculated. After weighing, 10 fruits were randomly taken from the infested fruits of each replicate, of each genotype and were brought into the laboratory, where they were observed individually under a microscope. The larvae, in each fruit, were counted and the number of larvae per fruit was calculated. The six genotypes were again grouped, by following the rating system, given by Nath (1966), to confirm their resistance level.

**Study of the biophysical fruit-traits of bitter-gourd genotypes:** Fifteen marketable fresh fruits (five fruits, per repeat, per genotypes) of each of the six genotypes, which were sown for rescreening purposes, were used to record data on the biophysical factors of resistance. Biophysical fruit-traits, like, fruit-length, fruit-diameter, numbers of the longitudinal ribs, thickness of the longitudinal ribs, depth of the longitudinal ribs, number of small ridges per cm<sup>2</sup>, depth of small ridges, pericarp-thickness and toughness of fruit were measured. Fruit-length, fruit-diameter, thickness of the longitudinal ribs, depth of longitudinal ribs, depth of small ridges and pericarp-thickness were measured with the help of vernier caliper from five different positions of each fruit. Number of longitudinal ribs per fruit and the number of small ridges per cm<sup>2</sup> were recorded visually. Fruit-toughness or firmness was measured with the help of a Penetrometer (tr. Italy, model No. 53205, serial No. 15527) on five different positions of each fruit, of each variety in the Post-Harvest Losses Laboratory, Ayub Agriculture Research Institute, Faisalabad, Pakistan.

**Statistical analysis:** The data collected on percentage fruit-infestation and larval-density per fruit, among the tested bitter-gourd genotypes, during preliminary screening and rescreening trials in the field at Harappa and Faisalabad, were analyzed through a Multivariate General Linear Model (MGLM) Technique (Tabachnick & Fidell, 2001), through Factorial ANOVA test; whereas, the biophysical fruit-traits, among tested genotypes of bitter-gourd under lab conditions, were analyzed through one way ANOVA technique, by using SPSS software (O'Connor, 2000): i) to determine either the differences in above mentioned parameters are significant or nonsignificant among tested genotypes and ii) to calculate means alongwith their standard deviations. The means of significant parameters, among tested genotypes, were compared by using Tukey's Honestly Significant Difference (HSD) tests for paired comparisons, after an analysis of variance (ANOVA) at a probability level of 5%. Regression and correlation between biophysical fruit-traits of bitter-gourd genotypes and fruit fly-related, biological parameters (percent fruit infestation and larval-density per fruit), were also established, using correlation analysis technique and step-wise multiple regression analysis at a 95% significance level.

## Results

**Preliminary field evaluation of bitter-gourd genotypes:** The percentage fruit-infestation (d.f = 12;  $F = 23.94$ ;  $p < 0.01$ ) and larval-density per fruit (d.f = 12;  $F = 19.07$ ;  $p < 0.01$ ), varied significantly in the bitter-gourd genotypes, tested during 2005 at Harappa and Faisalabad (Table 1). The larval-density per fruit ranged from 2.4 to 9.3 and 2.4 to 9.4 larvae per fruit, during the 2005, at Harappa and Faisalabad, respectively. The mean of larval-density per fruit, at both localities, was also found to be in the same range i.e., 2.4 to 9.35 larvae per fruit, being significantly lowest in resistant genotypes and highest in susceptible genotypes. The fruit infestation at Harappa ranged from 18.7 to 75.3%, while at Faisalabad, it ranged from 16.7 to 73.3%. The mean of fruit infestation, at both localities, was found to be in the range of 17.7 to 74.4%, being significantly lowest in resistant genotypes and highest in susceptible genotypes. The genotypes classified as resistant, moderately resistant and susceptible on the basis of percentage fruit infestation at Harappa also showed similar degree of resistance or susceptibility at Faisalabad. The level of fruit fly infestation and that of larval-density per fruit was found similar when compared with that of Faisalabad, across the genotypes. There was no change in the relative ranking of tested bitter-gourd genotypes at both localities. Col-II and Faisalabad-Long showed 17.7 and 19.65% fruit infestation, respectively and were ranked resistant genotypes. Col-Nankana Sahib, Col-I and GS-51, with 35-48.35% fruit infestation, showed moderately resistant reactions to melon fruit flies at both localities, whereas, Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F<sub>1</sub>, Janpuri, F<sub>1</sub>-484 and F<sub>1</sub>-485, which showed 54.3-74.4% fruit infestation, were ranked susceptible accessions (Table 1). The larval density per fruit had a significant positive correlation ( $r = 0.985$ ) with percentage fruit infestation. The larval-density per fruit increased with an increase in percentage fruit infestation. No significant differences were observed for number of larvae per fruit at both localities (Table 1).

**Table 1. Means  $\pm$  SD of the percentage fruit-infestation and larval-density, per fruit of the melon fruit fly, on different genotypes of bitter-gourd, during preliminary screening trails, at Harappa, Punjab, Pakistan in 2005.**

	Larval-density per fruit			% Fruit-infestation			Resistance category
	Harappa	Faisalabad	Mean	Harappa	Faisalabad	Mean	
	Col-II	2.4 $\pm$ 0.6 f	2.4 $\pm$ 0.8g	2.4 $\pm$ 0.25g	18.7 $\pm$ 3.1 e	16.7 $\pm$ 5.77g	
FSD-long	3.2 $\pm$ 0.3ef	3.7 $\pm$ 0.5fg	3.45 $\pm$ 0.42fg	19.3 $\pm$ 2.3 e	20 $\pm$ 0.0 fg	19.65 $\pm$ 1.85e	R
Col-Nankana Sahib	4.7 $\pm$ 0.4de	4.6 $\pm$ 0.7ef	4.65 $\pm$ 0.53ef	36.7 $\pm$ 2.3 d	33.3 $\pm$ 5.8ef	35.0 $\pm$ 4.21d	MR
Col-I	5.8 $\pm$ 0.7cd	5.9 $\pm$ 0.4de	5.85 $\pm$ 0.35de	44.7 $\pm$ 9.1 cd	46.7 $\pm$ 5.8de	45.7 $\pm$ 6.64cd	MR
GS-51	5.8 $\pm$ 0.8cd	6.1 $\pm$ 0.8de	5.95 $\pm$ 0.12de	46.7 $\pm$ 8.3 cd	50 $\pm$ 0.0 cd	48.35 $\pm$ 5.51cd	MR
Col-III	6.6 $\pm$ 1.2bc	6.5 $\pm$ 1.5cde	6.55 $\pm$ 0.25cde	55.3 $\pm$ 8.1 bc	53.3 $\pm$ 5.8bcd	54.3 $\pm$ 6.21bc	S
Col-Multan	7.3 $\pm$ 0.9bc	7.3 $\pm$ 1.8bcd	7.3 $\pm$ 1.1bcd	67.3 $\pm$ 16.2 ab	63.3 $\pm$ 15.3abc	65.3 $\pm$ 4.31ab	S
Col-Vehari	8.2 $\pm$ 2.7ab	8.2 $\pm$ 0.2abc	8.2 $\pm$ 1.35abc	71.3 $\pm$ 23.4 ab	66.7 $\pm$ 11.5ab	69 $\pm$ 15.75ab	S
Chaman	8.3 $\pm$ 1.9ab	8.4 $\pm$ 1.6abc	8.35 $\pm$ 2.1abc	72 $\pm$ 15.9 ab	70 $\pm$ 10a	71 $\pm$ 12.35a	S
Sunder-F1	8.2 $\pm$ 0.37ab	8.2 $\pm$ 0.6abc	8.2 $\pm$ 0.54abc	72.7 $\pm$ 6.1 a	70 $\pm$ 10a	71.35 $\pm$ 7.75a	S
Janpuri	7.5 $\pm$ 0.5 bc	8.6 $\pm$ 2.8ab	8.05 $\pm$ 1.15ab	73.3 $\pm$ 8.1 a	73.3 $\pm$ 20.8a	73.3 $\pm$ 14.56a	S
F1-484	8.0 $\pm$ 0.27ab	8.9 $\pm$ 0.7ab	8.45 $\pm$ 1.31ab	73.3 $\pm$ 9.0 a	73.3 $\pm$ 5.8a	73.3 $\pm$ 11.2a	S
F1-485	9.3 $\pm$ 0.97 a	9.4 $\pm$ 1.4a	9.35 $\pm$ 1.51a	75.3 $\pm$ 5.1 a	73.3 $\pm$ 11.5a	74.4 $\pm$ 9.25a	S

**Re-evaluation of six selected bitter-gourd genotypes:** Re-evaluation trails, conducted at Faisalabad and Harappa during 2006, showed that the percentage fruit-infestation (d.f = 5;  $F = 7980.4$ ;  $p < 0.01$ ) and larval-density per fruit (d.f = 5;  $F = 284.433$ ;  $p < 0.01$ ), varied significantly in the bitter-gourd genotypes at both localities. The larval-density per fruit ranged from 1.5 to 8.4 and 1.3 to 9.8 larvae per fruit, during the 2006, at Faisalabad and Harappa, respectively. The mean of larval-density per fruit, at both localities, was found to be in the range of 1.4 to 9.1 larvae per fruit, being significantly lowest in resistant genotypes and highest in susceptible genotypes. The fruit infestation at Harappa ranged from 17.9 to 77.87%, while at Faisalabad, it ranged from 17.5 to 78.5%. The mean of fruit infestation, at both localities, was found to be in the range of 17.7 to 78.18%, being significantly lowest in resistant genotypes and highest in susceptible genotypes. The genotypes classified as resistant, moderately resistant and susceptible on the basis of percentage fruit infestation at Harappa also showed similar degree of resistance or susceptibility at Faisalabad. The level of fruit fly infestation and that of larval-density per fruit was found similar when compared with that of Faisalabad, across the genotypes. No change was observed in the relative ranking of tested bitter-gourd genotypes at both localities, again during 2007. Col-II and Faisalabad-Long showed 17.7 and 19.55% fruit infestation, respectively and were again ranked resistant genotypes. Col-I and Col-Nankana Sahib, with 48.65-49.3% fruit infestation, showed moderately resistant reactions to melon fruit flies at both localities, during 2007, whereas, Chaman and Col-Vehari showed 76.1-78.1% fruit infestation and were again ranked susceptible accessions (Table 2). The larval density per fruit again had a significant positive correlation ( $r=0.992$ ) with percentage fruit infestation. The larval-density per fruit increased with an increase in percentage fruit infestation. No significant differences were observed for number of larvae per fruit at both localities (Table 2).

**Biophysical fruit-traits of the re-evaluated bitter gourd genotypes:** The fruit-length, fruit-diameter, number of longitudinal ribs/fruit and number small ridges/cm<sup>2</sup> ranged from 8.3 to 12.1 cm, 3.02 to 4.41 cm, 8.4 to 9.7 ribs/fruit and 9.4 to 13.7 ridges/cm<sup>2</sup>, respectively, being significantly lowest in resistant and highest in susceptible genotypes. However, the fruit toughness, height of small ridges, height of longitudinal ribs and pericarp thickness ranged from 4.7 to 7.2 kg/cm<sup>2</sup>, 2.5 to 3.9 mm, 3.1 to 7.0 mm and 3.9 to 6.2 mm, respectively, being significantly highest in resistant and lowest in susceptible genotypes (Table 3)

**Influence of biophysical fruit-traits on resistance of bitter-gourd genotypes to melon fly:** Regression and correlation analysis between biophysical fruit-traits and percentage fruit infestation, revealed that the fruit-length ( $r=0.987$ ;  $P=0.000$ ); fruit-diameter ( $r=0.890$ ;  $P=0.017$ ); number of longitudinal ribs ( $r=0.965$ ;  $P=0.002$ ) and number small ridges per cm<sup>2</sup> ( $r=0.934$ ;  $P=0.006$ ), had a significant positive correlation; whereas, the fruit-toughness ( $r= -0.939$ ;  $P=0.005$ ); height of longitudinal ribs ( $r= -0.964$ ;  $P=0.002$ ); depth of small ridges ( $r= -0.989$ ;  $P=0.000$ ) and pericarp/flesh-thickness ( $r= -0.951$ ;  $P=0.003$ ), had a significant negative correlation with the percentage fruit-infestation. Similarly, the fruit-length ( $r=0.990$ ;  $P=0.000$ ); fruit-diameter ( $r=0.885$ ;  $P=0.019$ ); number of longitudinal ribs ( $r=0.945$ ;  $P=0.004$ ) and number small ridges per cm<sup>2</sup> ( $r=0.922$ ;  $P=0.009$ ), had a significant positive correlation; whereas, the fruit-toughness ( $r=-0.968$ ;  $P=0.002$ ); height of longitudinal ribs ( $r=-0.977$ ;  $P=0.001$ ); depth of small ridges ( $r= -0.983$ ;  $P=0.000$ ) and pericarp/flesh-thickness ( $r= -0.982$ ;  $P=0.000$ ), had a significant negative correlation with the larval-density per fruit (Table 4).

**Table 2. Means  $\pm$  SD of the percentage fruit-infestation and larval-density, per fruit, on different bitter-gourd genotypes, after rescreening, at Faisalabad and Harappa, during 2006.**

bitter-gourd genotypes	Larval-density/fruit			% fruit-infestation			Resistant category
	Faisalabad	Harappa	Mean	Faisalabad	Harappa	Mean	
ol-II	1.5 $\pm$ 0.66d	1.3 $\pm$ 0.30c	1.4c	17.5 $\pm$ 1.1c	17.9 $\pm$ 0.52d	17.7c	R
SD-long	2.2 $\pm$ 0.81cd	2.3 $\pm$ 0.30c	2.25c	19.5 $\pm$ 1.15c	19.6 $\pm$ 0.17d	19.55c	R
ol-I	4.2 $\pm$ 1.1bc	4.7 $\pm$ 0.75b	4.45b	48.7 $\pm$ 0.9b	48.6 $\pm$ 1.35c	48.65b	MR
ol-Nankana Sahib	5.3 $\pm$ 1.0b	5.8 $\pm$ 0.68b	5.55b	49.5 $\pm$ 0.85b	49.17 $\pm$ 0.83c	49.3b	MR
haman	8.3 $\pm$ 1.06a	9.1 $\pm$ 1.04a	8.7a	76.6 $\pm$ 1.2a	75.67 $\pm$ 0.51b	76.1a	S
ol-Vehari	8.4 $\pm$ 1.05a	9.8 $\pm$ 0.041a	9.1a	78.5 $\pm$ 0.8a	77.87 $\pm$ 0.55a	78.18a	S

**Table 3. Means  $\pm$  SD of different of biophysical fruit-traits of the rescreened genotypes of bitter-gourd at 95% C.I., in 2006.**

bitter-gourd genotypes	FL	FD	FT	NLRbs	NSRgs	DSRgs	HLRbs	PTh
ol-II	8.3 $\pm$ 0.21a	3.02 $\pm$ 0.04a	7.2 $\pm$ 0.02a	8.4 $\pm$ 0.2a	9.4 $\pm$ 0.18a	3.9 $\pm$ 0.13a	7.0 $\pm$ 0.19a	6.2 $\pm$ 0.01f
SD-long	8.9 $\pm$ 0.14a	3.12 $\pm$ 0.04a	6.4 $\pm$ 0.05b	8.4 $\pm$ 0.00a	9.7 $\pm$ 0.50a	3.8 $\pm$ 0.55a	5.7 $\pm$ 0.02b	5.5 $\pm$ 0.08e
ol-I	10.3 $\pm$ 0.22b	3.18 $\pm$ 0.13a	5.8 $\pm$ 0.04c	8.7 $\pm$ 0.23a	10.3 $\pm$ 0.41a	3.0 $\pm$ 0.07b	4.9 $\pm$ 0.10c	4.9 $\pm$ 0.10d
ol-Nankana Sahib	10.7 $\pm$ 0.37b	3.72 $\pm$ 0.05b	5.3 $\pm$ 0.21d	9.2 $\pm$ 0.35ab	10.4 $\pm$ 0.11a	3.1 $\pm$ 0.03b	4.7 $\pm$ 0.24c	4.7 $\pm$ 0.03c
haman	11.8 $\pm$ 0.5c	3.82 $\pm$ 0.05b	4.9 $\pm$ 0.03e	9.6 $\pm$ 0.35b	13.2 $\pm$ 0.09b	2.3 $\pm$ 0.02c	2.7 $\pm$ 0.13d	4.3 $\pm$ 0.03b
ol-Vehari	12.1 $\pm$ 0.03c	4.41 $\pm$ 0.12c	4.7 $\pm$ 0.03f	9.7 $\pm$ 0.23b	13.7 $\pm$ 1.1b	2.5 $\pm$ 0.04 bc	3.1 $\pm$ 0.05e	3.9 $\pm$ 0.03a

Means with similar letters do not differ significantly, as determined by Tukey HSD Test, at 0.05 level.

**L:** Fruit-length (cm); **FD:** Fruit-diameter (cm); **FT:** Fruit-toughness (Kg/cm<sup>2</sup>); **NLRbs:** No. of the Longitudinal ribs/fruit; **NSRgs:** No. of the small ridges, per cm<sup>2</sup>; **DSRgs:** Depth of the small ridges (mm); **HLRbs:** Height of the longitudinal ribs (mm); **PTh:** epicarp/flesh-thickness (mm).

**Table 4. The correlation coefficients (without brackets) of the different biophysical fruit-traits of the rescreened genotypes of bitter-gourd with the percent fruit-infestation and larval-density per fruit.**

	FL	FD	FT	NLRbs	NSRgs	DSRgs	HLRbs	PTh	% fruit-infestation
D	.863*								
T	-.965**	-.882*							
	(.002)	(0.020)							
JLRbs	.955**	.948**	-.914*						
	(.003)	(.004)	(.011)						
WLRbs	.791ns	.918**	-.874*	.806ns					
	(.061)	(.010)	(.023)	(.053)					
JSRgs	.906*	.903*	-.840*	.933**					
	(.013)	(.014)	(.036)	(.006)					
WSRgs	-.986**	-.816*	.921**	-.928**	-.908*				
	(.000)	(.047)	(.009)	(.008)	(.012)				
ILRbs	-.985**	-.848*	.951**	-.931**	-.930**	.967**			
	(.000)	(.033)	(.003)	(.007)	(.007)	(.002)			
rTh	-.955**	-.861*	.973**	-.884*	-.878*	.942**	.953**		
	(.003)	(.028)	(.001)	(.019)	(.021)	(.005)	(.003)		
% fruit-infestation	.987**	.890*	-.939**	.965**	.934**	-.989**	-.964**	-.951**	
	(.000)	(.017)	(.005)	(.002)	(.006)	(.000)	(.002)	(.003)	
arvae/fruit	.990**	.885*	-.968**	.945**	.922**	-.983**	-.977**	-.982**	.992**
	(.000)	(.019)	(.002)	(.004)	(.009)	(.000)	(.001)	(.000)	(.000)

Correlation is significant at 0.05 level (2-tailed). \*\* Correlation is significant at 0.01 level (2-tailed). <sup>ns</sup> Correlation is non-significant at 0.05 level (2-tailed); values in brackets are the P<sub>s</sub>

**L**: Fruit-length (cm); **FD**: Fruit-diameter (cm); **FT**: Fruit-toughness (Kg/cm<sup>2</sup>); **NLRbs**: No. of the longitudinal ribs/fruit; **NSRgs**: No. of the small ridges, per cm<sup>2</sup>; **DSRgs**: Depth of the small ridges (mm); **HLRbs**: Height of the longitudinal ribs (mm); **PTh**: Pericarp/flesh-thickness (mm).



**Table 5. Step-wise regression models, showing coefficient of determination ( $100R^2$ ) values of different biophysical fruit-traits for the number of larvae per fruit (a) and percentage fruit infestation (b).**

	$100R^2$	Role of individual fruit-trait
<b>(a) Number of larvae/fruit</b>		
$Y = 24.29 - 1.55X_1$	9.56	9.56
$Y = 17.6 - 4.5X_1 + 10.26X_2$	31.9	22.34
$Y = 67.22 - 0.24X_1 - 3.6X_2 - 7.69X_3$	88.1	49.2
$Y = -38.80 + 0.64X_1 - 5.72X_2 - 3.4X_3 + 8.9X_4$	99.67	11.57
$Y = -39.88 + 0.837X_1 - 6.61X_2 - 4.93X_3 + 9.56X_4 + 1.05X_5$	99.91	0.24
$Y = -31.11 + 0.55X_1 - 3.29X_2 - 5.6X_3 + 4.16X_4 + 2.56X_5 + 2.49X_6$	99.99	0.08
$Y = -29.17 + 0.329X_1 - 2.63X_2 - 5.21X_3 + 3.93X_4 + 2.73X_5 + 2.5X_6 - 0.94X_7$	99.99	0.0
$Y = -24.08 + 0.587X_1 - 2.47X_2 - 8.41X_3 + 1.51X_4 + 3.47X_5 + 3.73X_6 + 0.3X_7 + 2.19X_8$	100	0.01
<b>(b) Percentage fruit infestation</b>		
$Y = 74.41 - 1.7X_1$	0.64	0.64
$Y = 45.45 - 14.6X_1 + 4.6X_2$	23.86	23.22
$Y = 269.3 + 4.8X_1 - 18X_2 - 34.7X_3$	87.26	63.4
$Y = -110.3 + 7.9X_1 - 25.7X_2 - 19.3X_3 + 31.9X_4$	95.49	8.23
$Y = -122.3 + 10.1X_1 - 35.6X_2 - 36.2X_3 - 39.2X_4 + 11.6X_5$	97.07	1.58
$Y = 57.83 + 4.4X_1 + 33X_2 - 50.1X_3 - 72X_4 + 42.8X_5 + 51.1X_6$	98.94	1.87
$Y = -36.51 + 15.6X_1 + 0.41X_2 - 69.2X_3 - 60.2X_4 + 35X_5 + 50.5X_6 + 45.5X_7$	99.7	0.76
$Y = -23.3 + 5.61X_1 - 5.61X_2 + 54.3X_3 + 33.1X_4 + 6.67X_5 + 3.07X_6 - 2.0X_7 - 84.6X_8$	100	0.3

$X_1$ : Fruit-length (cm);  $X_2$ : Fruit-diameter (cm);  $X_3$ : Fruit-toughness (Kg/cm<sup>2</sup>);  $X_4$ : No. of the longitudinal ribs/fruit;  $X_5$ : Height of the longitudinal ribs (mm);  $X_6$ : No. of the small ridges, per cm<sup>2</sup>;  $X_7$ : Depth of the small ridges (mm);  $X_8$ : Pericarp/flesh-thickness (mm).

Step-wise multiple regression analysis indicated that the biophysical fruit-traits explained 100% of the total variation in fruit fly infestation. Stepwise regression analysis also indicated that fruit-length, fruit-diameter, fruit-toughness and number of longitudinal ribs explained 95.49% of the total variation in fruit fly infestation. The maximum variation in fruit infestation was explained by fruit toughness (63.4%) followed by fruit-diameter (23.22%) and number of longitudinal ribs (8.23) and these can be used as marker traits to select for resistance to melon fruit fly in bitter gourd; whereas, rest of the biophysical fruit-traits explain less than 2% variation in the fruit infestation (Table 5a). Similarly, the biophysical traits explained 100% of the total variation in larval-density per fruit. The fruit-length, fruit-diameter, fruit-toughness and number of longitudinal ribs explained 99.67% of the total variation in the larval-density per fruit. The maximum variation in the larval-density per fruit was explained by fruit toughness (49.2%), followed by fruit-diameter (22.34%), number of longitudinal ribs (11.57) and fruit-length (9.56%) and these can be used as marker traits to select for resistance to melon fruit fly in bitter gourd; whereas, rest of the biophysical fruit-traits explain less than 1% variation in fruit infestation (Table 5b).

## Discussion

Plant genotypes, either due to the environmental stress or genetic make up, possess physiological and biochemical variations, which alter the nutritional values (primary metabolites) for herbivores (Eckey-Kaltenbach *et al.*, 1994; Mısırlı *et al.*, 2000; Siemens *et al.*, 2002; Goncalves-Alvim *et al.*, 2004; Masood *et al.*, 2005; Shad *et al.*, 2006; Zakir *et al.*, 2006). Antixenosis, which refers to the potential plant-characteristics/traits, either allelochemic or morphological, that impair or alters insect behavior towards the host-preference (Carter, 1927; Mumford, 1931; Rhoades, 1983; Edelstein-Keshet, 1986; Harrison & Karban, 1986; Edelstein-Keshet & Rausher, 1989; Adler & Karban, 1994; Morris & Dwyer, 1997; Thaler, 1999; Underwood, 1999; Afzal *et al.*, 2009), in such a way, as to lessen chances of insects, using a host plant for oviposition (Painter, 1951; Chapman *et al.*, 1983; Valencia, 1984; Karban, *et al.*, 1997), food, damage or shelter (Painter, 1951; Chapman *et al.*, 1983; Dabrowski & Kidiavai, 1983; Woodhead & Taneja, 1987; Sharma & Nwanze, 1997). Preliminary screening and rescreening trails, conducted at Harappa and Faisalabad, also showed significant differences in the genotypic resistance/susceptibility, for fruit-infestation and larval-density of melon fruit fly, in bitter-gourd genotypes. Dhillon *et al.*, (2005), Srinivasan (1991), Thakur *et al.*, (1992, 1994, 1996) and Tewatia *et al.*, (1997) also reported significant differences in the genotypic resistance/susceptibility for fruit-infestation and larval-density of melon fruit fly in bitter-gourd genotypes. These variations can be attributed to several, environmentally or genetically, induced physiological and biochemical variations in plant traits (Eckey-Kaltenbach *et al.*, 1994; Mısırlı *et al.*, 2000; Siemens *et al.*, 2002; Theis & Lerda, 2003; Goncalves-Alvim *et al.*, 2004).

Preliminary screening trials, conducted at Harappa and Faisalabad, revealed non-significant differences for fruit-infestation and larval-density, per fruit, between localities. However, rescreening trials showed significant differences for larval-density. These differences can be attributed to variations in the genotypic resistance/susceptibility responses of bitter-gourd genotypes and/or population build-up of melon fruit fly, at both localities, which are directly or indirectly influenced by the year-wise variations in abiotic factors, like, temperature, relative humidity, rain fall etc., and plantation activity.

Su (1986) & Lee *et al.*, (1992) documented the similar reasons behind the fluctuation in population-density of *Bactrocera cucbitae*, in Taiwan. Percentage fruit-infestation and larval-density, per fruit, were found significantly lower in resistant genotypes and higher in susceptible genotypes of bitter-gourd. These results are highly in agreement with those of Dhillon *et al.*, (2005), who reported a significantly lower percentage fruit-infestation and larval-density, per fruit, in wild genotypes (resistant) and higher in cultivated genotypes (susceptible) of bitter-gourd. Col-II and FSD-long, were found resistant genotypes to melon fruit flies, whereas, Col-Nankana sahib, Col-I and GS-51 were seen to be moderately resistant and Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F1, Janpuri, F1-484 and F1-485 were susceptible to the melon fruit fly. No literature, on the resistance/susceptibility of these bitter-gourd genotypes for melon fruit fly, is available; hence these results can not be compared or correlated with any research report.

The bitter-gourd genotypes in India, wild and cultivated, possess significant variations for their biophysical fruit-traits (Srinivasan, 1991; Thakur *et al.*, 1992, 1994, 1996; Tewatia *et al.*, 1997; Dhillon *et al.*, 2005). In these findings, biophysical fruit-traits were also found significantly different among bitter-gourd genotypes. Similar results were documented by Dhillon *et al.*, (2005). Fruit-length; fruit-diameter; number of longitudinal ribs, per fruit; width of longitudinal ribs and number of small ridges, per cm<sup>2</sup>, were significantly lower in resistant and higher in susceptible genotypes. However, the fruit-toughness, height of longitudinal ribs, depth of small ridges and pericarp/flesh-thickness, were significantly higher in resistant varieties and lower in susceptible genotypes. Contrary to our findings, Dhillon *et al.*, (2005) concluded that the number of ridges, per cm<sup>2</sup> and the depth of ribs were higher in resistant genotypes. They also reported that fruit-thickness was higher in resistant genotypes, which is inconsistency with the results of these studies. In these findings, fruit-length; fruit-diameter; number of longitudinal ribs per fruit; width of longitudinal ribs; number of small ridges per cm<sup>2</sup>; fruit-toughness; height of longitudinal ribs; depth of small ridges and pericarp/flesh-thickness, ranged from 8.3 to 12.1cm, 3.02 to 4.4 cm, 8.4 to 9.7, 0.33 to 0.69 cm, 9.4 to 13.7, 4.7 to 7.2 Kg/cm<sup>2</sup>, 2.7 to 7.0 mm, 2.3 mm to 3.9 mm and 3.9 to 6.2 mm, respectively; whereas, Dhillon *et al.*, (2005) reported that the fruit-length; fruit-diameter; number of ridges per cm<sup>2</sup>; fruit-toughness; depth of ribs and pericarp/flesh-thickness ranged from 2.23 to 15.29 cm, 1.69 to 4.06 cm, 17.8 to 118.13, 7.35 to 10.73 Kg/cm<sup>2</sup>, 1.37 to 8.61 mm and 2.39 to 6.28 mm, respectively. These variations in measurements of biophysical fruit-traits may be attributed to differences in the tested genotypes and/or stage of the fruits selected for measuring these traits, as reported by Kumara *et al.*, (2006).

The insects are attracted to or repelled by a plant, due to a variety of physio-morphic plant characteristics (Karban *et al.*, 1997; Ernest, 1989), such as, its shape, size (Prokopy & Owens, 1983; Langan *et al.*, 2001), colour (Prokopy & Owens, 1983; Hirota & Kato, 2001), surface texture (Pritchard, 1969; Spencer *et al.*, 1999), presence of trichomes and wax crystals on the surface, the thickness and toughness of the tissue (Ernest, 1989), tough vascular bundles (Howe, 1949), length and diameter of the fruits, depth of ribs, flesh-thickness, intensity of ribs and fruit-toughness (Boller & Prokopy, 1976; Dhillon *et al.*, 2005). The present findings also showed highly significant differences in percentage fruit-infestation and larval-density per fruit among tested genotypes. Dhillon *et al.*, (2005), who evaluated seventeen: six wild and eleven cultivated genotypes of bitter-gourd for fruit-infestation and larval-density/fruit, also reported the same results. The percentage fruit-infestation (17.7-74.4%) and larval-density per fruit (2.4-9.35

larvae/fruit) were significantly lower in resistant genotypes and higher in highly susceptible genotypes. These results are in agreement with the reports of Dhillon *et al.*, (2005), that wild-accessions (resistant) exhibited 8.3-12.6% and susceptible accessions showed 65.5-69% fruit-infestation, whereas, the wild accessions (resistant) exhibited 3.8-5.1 larvae/fruit and susceptible accessions showed 7.8-8.5 larvae/fruit. The variations between these findings and findings reported by Dhillon *et al.*, (2005) on fruit-infestation and larva- density/fruit may be attributed to differences in genetic makeup of the tested genotypes, agronomic practices conducted and ecological conditions of localities of countries, where these trials were conducted. As a result of limited literature on such aspect, present finding can be compared with the findings of only few researchers, who documented such interaction between plants or plant parts and insects other than that studied in the present research. For example, Howard & Kenney (1987) determined that 'Golden Star' was less susceptible to Caribbean fruit fly than 'Arkin' or 'Fwang Tung' based on the number of eggs, oviposited in harvested fruits.

The variations in fruit-infestation and larval-density per fruit, alongwith ecological conditions and physiological status of insects, can be correlated with the biophysical and biochemical fruit-traits (Ponti, 1977). Morphological/physical fruit-traits, like, tough vascular bundles (Howe, 1949), length and diameter of the fruits, depth of ribs, flesh-thickness, intensity of ribs fruit-toughness etc., interfere with the feeding and oviposition by the fruit flies (Boller & Prokopy, 1976; Dhillon *et al.*, 2005). Physical fruit-properties, such as size, shape and colour are the initial stimuli that stimulate locomotory activity of fruit flies for host location and finally their orientation to the potential ovipositional site (Boller & Prokopy, 1976; Prokopy & Owens, 1983); whereas, pericarp/skin-toughness and the surface texture of the fruit, determine the acceptability of the fruit for fruit fly oviposition (Boller & Prokopy, 1976; Dhillon *et al.*, 2005). Present findings indicate that fruit-length, fruit-diameter, number of longitudinal ribs and number of small ridges per  $\text{cm}^2$  had a significant positive correlation, whereas, fruit-toughness, height of longitudinal ribs, depth of small ridges and pericarp/flesh-thickness had a significant negative correlation with the percentage fruit-infestation and larval-density, per fruit. The findings of Dhillon *et al.*, (2005) that fruit-diameter and fruit-length had a positive and significant, whereas, toughness of fruit had a negative and significant correlation with the fruit-infestation and larval-density, per fruit, are in agreement with the present findings. However, their findings that depth of ribs and flesh thickness, were positively and significantly; whereas, number of ridges/ $\text{cm}^2$  were negatively correlated with the fruit-infestation and larval-density, per fruit, are not inconsistent with the present findings. The number of small ridges/ $\text{cm}^2$  should influence the fruit-infestation and larval-density/fruit positively, rather than negatively, because, a large number of small ridges/ $\text{cm}^2$  enhances the surface roughness, which ultimately increases the gripping capability of fruit flies, during oviposition, hence, it increases the chances of heavy fruit-infestation and *vice versa*. Similarly, more depth/height of longitudinal ribs decreases the chances of ovipositor to reach the fruit skin and more toughness of fruit decreases the chances of ovipositor insertion excessively into the fruit skin. Pritchard (1969) also reported that, if the preferred fruit is found to be too smooth, oily and tough, the fly may leave the fruit, as the ovipositor can neither grip nor penetrate the fruit. Very limited literature is available on the correlation between biophysical fruit-traits of bitter-gourd and melon fruit fly infestation. However, present findings can be compared with the findings of some researchers, who documented such correlations between plants or plant parts and insects other than that studied in the present research. For example, Jaiswal *et al.*, (1990)

& Tewatia *et al.*, (1997), also indicated that certain fruit characters, such as, fruit-length and diameter, affect the percentage fruit-infestation which increases with an increase in fruit-length and diameter. *Bactrocera cucurbitae* (Coquillett) and *Bactrocera dorsalis* (Hendel), oviposit, in breaks, on the skin (Prokopy & Koyama, 1982). Tough rind, in the fruits of *Cucumis callosus*, inhibited the egg-lying by melon fruit fly to 17.77%. However, egg lying was stimulated to 87.33% in fruits of the susceptible variety, Delta Gold, having fruits with soft rind (Chelliah & Sambandam, 1971). Similar results have been reported by Pal *et al.*, (1984), who found thick and tough rind-fruits of IHR 89 and IHR 213 genotypes to be resistant to melon fruit fly. According to Mumford, (1931), thin-skinned varieties of citrus, like, tangerines, *Citrus fiabilis* var. *deliciosa* and Satsumas, *C. nobilis* var. *ulzshiu* suffered most from the attacks of plant-bugs, as, *Nezara viridula* Linn. However, oranges, *C. silzelzsis* and grape-fruit, *C. gralzdiz*, having a thick skin, were rarely attacked. Similarly, thick-skinned varieties of apple, papaya and melon are not so readily attacked by the apple maggot, *Rhagoletis ponzonella* (Walsh), papaya fruit-fly, *Poxotrypana curvicauda* Gerst., and the melon-fly, *Bactrocera (Dacus) cucurbitae* (Coq.), respectively (Mumford, 1931). In conclusion, Col-II and Faisalabad-Long, which were identified as source of resistance for melon fruit fly, *B. cucurbitae* can be used in IPM program for melon fruit fly and breeding program of bitter gourd. Among determined biophysical fruit traits, fruit toughness, fruit-diameter and number of longitudinal ribs can be used as marker traits to induce resistance against melon fruit fly in the cultivars of bitter gourd.

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