

Ovipositional Preference and Performance of Oriental Fruit Fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) on Some Commercial Citrus Cultivars

Muhammad Ismail<sup>1\*</sup>, Abu Bakar Muhammad Raza<sup>1</sup>, Muhammad Zeeshan Majeed<sup>1</sup>, Muhammad Anjum Aqueel<sup>2</sup>

<sup>1</sup>Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan

<sup>2</sup>Department of Entomology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

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# Abstract

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a destructive pest of horticultural crops in Pakistan. The present research goal was to evaluate the most suitable host choice for oviposition and offspring performance of *B. dorsalis* among different citrus cultivars in the laboratory under no-choice and choice tests. Based on pupal recovery, *B. dorsalis* showed maximum infestation on *Citrus reticulata* (mandarin) followed by *Citrus sinensis* (sweet orange) both under choice and no-choice tests compared to other cultivars. Findings showed that *C. reticulata* was the most preferred host in terms of oviposition choice with an average pupal recovery (10.67%), followed by *C. sinensis* with an average pupal recovery (7.50%) under the choice test. While *Citrus aurantifolia* (lime) followed by *Citrus paradisi* (grapefruit) showed significant deformities under both tests, making them unfavorable hosts for *B. dorsalis*. In case of peel thickness, *C. aurantifolia* showed maximum thickness (0.74 cm) followed by *C. aurantium* (0.58 cm), while *C. paradisi* had the least peel thickness (0.43 cm). In case of physiochemical parameters, a significant difference (P < 0.05) was observed for acidity (%) values. However, there was no relationship between these characters on the ovipositional behaviour of *B. dorsalis*. Only the peel thickness (R<sup>2</sup> = 56.2%), fruit weight (R<sup>2</sup> = 54.4%) and fruit diameter (R<sup>2</sup> = 60.7%) had a small role in the ovipositional behaviour of *B. dorsalis*.

\* Corresponding Author: Muhammad Ismail 🖂 ismailbilu2643@gmail.com

## Introduction

Tropical fruit flies (Diptera: Tephritidae) are essential agricultural pests in Pakistan (Abdullah et al., 2002; Sarwar et al., 2013; Sarwar, 2015;), causing direct losses at the farm level as well as subsidiary damages to exporters and traders (Staub et al., 2008; Shelly et al., 2011; Sandeep and Desraj, 2016). Strict quarantine restrictions are imposed by many countries to curtail their entry as well (Aluja, 2003; Clarke et al., 2005; Follett and Neven, 2006). Fruit damage without control has been weighed as 24% in Pakistan (Stonehouse et al., 1998). Bactrocera dorsalis Hendel (Diptera: Tephritidae) is one of the significant pests of fruits across Asia and the Pacific islands, attacking and depositing their eggs in various fruits and vegetables the flesh of which is then consumed by their maggots (Katsoyannos et al., 1999; Stark et al., 2004; Verghese et al., 2004; Clarke et al., 2005).

Many tephritid species prefer to lay eggs in soft fruit and in cracks, wounds and cavities (Balagawi *et al.*, 2005; Papachristos and Papadopoulos, 2009). Once the injury is done on peel, the female deposits eggs into the fruit, where the larvae hatch and feed on the pulp of fruit (Prokopy and Koyama, 1982). Direct crop losses are initiated by feeding by maggots and fruit drop (Lux *et al.*, 2003; Ekesi *et al.*, 2009), but substantial indirect losses result from quarantine limitations on possibly infested fruits (Heather and Hallman, 2008).

For developing successful management techniques, it is imperative to understand the insect-plant relationship concerning life-history traits and host preference (Rwomushana *et al.*, 2008). Besides, a preference-performance hypothesis is also critical in the host patterns of a specific insect pest (Muthuthantri and Clarke, 2012). Females find and evaluate larval hosts using olfactory, visual and contact cues including the colour, size, shape and smell of fruit, twig and foliage of host plants (Katsoyannos *et al.*, 2011; Prokopy and Boller, 2011). Furthermore, the nutritional value of fruit can influence the infestation level (Drew *et al.*, 2003; Bush and Butlin, 2004; Brévault and Quilici, 2007).

Citrus fruits exhibit a few resistance mechanisms that reduce survival, fecundity and longevity of the attacking tephritids (Nikos et al., 2015; Papadopoulos et al., 2015). Fruit fly females force their ovipositor into citrus fruit peels, forming oviposition cavities into which eggs are deposited (Prokopy and Boller, 2011; Dias et al., 2018). Therefore, citrus fruit peel is the first barrier that newly hatched larvae face (Rafiq et al., 2016; Simas et al., 2017). As larvae penetrate through the peel to more nutritious fruit pulp, they must overcome the toxic effects of flavedo chemical substances, mainly essential oils (Salvatore et al., 2004; Papachristos et al., 2008; Muthuthantri and Clarke, 2012; Dias et al., 2018). Moreover, the physiochemical characteristics of host fruits, such as pH and soluble solid contents, may also affect the performance of immature stages of fruit flies (Ioannou et al., 2012; Papanastasiou et al., 2020). Different citrus varieties and cultivars have been found to have variable effects on several life-history parameters of immature stages of fruit flies (Papadopoulos, 2006) including survival rates and developmental times. Nevertheless, only a few studies compared the effects of different physical and chemical properties of different citrus varieties or cultivars on the biological parameters of the immature stages of fruit flies.

Keeping in view the economic importance of *B*. *dorsalis*, the current study was designed to find detailed information regarding the physicochemical properties of different citrus cultivars and their effect on life-history traits of *B*. *dorsalis* under laboratory conditions.

#### Materials and methods

#### Rearing of fruit flies

The newly hatched culture of *B. Dorsalis* was taken from the Department of Pest Warning and Quality Control, Sargodha, Punjab, Pakistan and was further established in the research lab at the College of Agriculture, University of Sargodha at  $27 \pm 1^{\circ}$ C,  $60 \pm 5\%$  relative humidity and 16h:10h light and dark photoperiod. Insect culture was reared on an artificial diet comprising of egg yolk, sugar, honey, yeast, syrup vitamin B complex in the ratio of 2:4:8:2:2:1, respectively. These ingredients were mixed via an electric blender to make a paste and were kept in a freezer for subsequent use. Fifty newly emerging pairs of *B. dorsalis* adults from stock culture were sexed and transferred into a new rearing cage ( $30 \times 30 \times 30$  cm). The adults were fed with water soaked on sponge and sugar cubes with a mixture of yeast extract and sugar at a ratio of 3:1. Female adults of 21-days age were only used in the experiment, as this is the optimum age for them to oviposit eggs (Rattanapun *et al.*, 2009).

## Fruit hosts

The fruits of five citrus cultivars viz., mandarin (Citrus reticulata Blanco), sweet orange (Citrus sinensis (L.) Osbeck, grapefruit (Citrus lime (Citrus paradisi Macfad), aurantifolia (Christm), bitter orange (Citrus aurantium L.) were selected to investigate the ovipositional preference and efficiency of *B*. dorsalis. The fruits were obtained from a citrus orchard located at Lahore Road, Sargodha (32°4'56.878" N and 72°40'8.86" E). The orchard was free from any pesticide application during the collection of fruit samples. The collected fruits were packed in plastic containers and brought to the laboratory, where they were individually washed and labelled according to the fruit cultivar.

#### Host preference

For investigating the preference and efficiency of selected citrus cultivars, choice and no-choice tests were carried out. The experiments were consisted of five treatments and there were three replications of each treatment.

## Choice test

Three fruits of each cultivar are offered collectively as a free choice in a single cage ( $30 \times 30 \times 30$  cm) arranged at a distance of 10 cm each. The experimental cage was divided equally into nine subunits and each unit held three fruits of each cultivar. The lid of the plastic container was cut in the middle and replaced with a muslin cloth for ventilation. A small hole was perforated on the container side so that space was created for introducing a fly into the container. Ten pairs of flies from the established culture were released into the cage through the hole before it was covered with a sponge. It was believed that the presence of males could impact female oviposition behaviour. The fly was fed with water soaked on sponge, sugar cubes, and yeast extract and sugar mixture at the ratio of 3:1.

The flies and the fruits were left in the container for 24 h. After 24 h, fruits were removed and placed individually in separate plastic containers containing sterilized and fine dust and incubated for 14 days until all larvae had pupated. The recovered pupae from each fruit were counted and then transferred into small plastic cups for adult emergence. The offspring parameters recorded were; 1) pupae formed, 2) pupal weight, 3) deformity, 4) percentage of adult's emergence, and 5) sex ratio.

#### No-choice tests

In the no-choice test, three fruits of each citrus cultivar were placed individually in a round and transparent plastic container (24 × 10 cm). One pair of flies was released at the center of the cage. The fly was fed with water soaked on sponge, sugar cubes, and a mixture of yeast extract and sugar at the ratio of 3:1. The flies were exposed to fruits for 24 hours, whereas the fruits were removed and placed individually in separate plastic containers containing sterilized and fine dust and incubated for 14 days until all larvae had pupated. The offspring parameters recorded were the same as in the choice experiment. All the experiments (no-choice and choice) were repeated three times and were conducted under laboratory conditions 27 ± 1°C, 60 ± 5% relative humidity, and 16h:10h light and darkness.

### Physiochemical fruit characters

The following parameters determined the characteristics of different citrus cultivars as peel

thickness, fruit diameter, fruits weight (g), total soluble solids (TSS; %) and acidity (%). Fruit weight (20 fruits of each cultivar) was measured precisely on a digital balancing machine (NoEnName\_Null, JA4000C). The means of measurement of the fruits were recorded. Fruit peel thickness (20 fruit for each cultivar) was measured with the help of Mitutuyo Vernier Caliper LCD Model Number: 500-196.

The fruit peel was removed, a piece of peel was placed between the two measuring jaws, and the average values were recorded. For diameter measurement, all fruits sampled for every treatment were individually measured using Mitutuyo Vernier Caliper LCD Model Number: 500-196. The same sample fruits were tested for total soluble solids (TSS = °Brix). The TSS of the citrus flesh was determined from the juice of the squeezed fresh fruit samples using a digital pocket refractometer (Atago® 3810 (PAL-1). In the juice, soluble solids content (SSC) was calculated with a refractometer (RX-5000a- Atago®). Four millilitres of juice was diluted with 16 ml of distilled water and, in this dilution, pH was measured with a pH meter (pH-1 SMA LG-PreSens) and titrated with 0.1 N NaOH until pH reached 8.2.

### Statistical analysis

The data obtained on the hosts attacked by flies with variable levels of infestations (number of fruit fly larvae infesting individual host) in all citrus cultivars were subjected to analysis of variance (ANOVA) using Statistix 8.1 statistical package. The treatment means were compared using the least significant differences (LSD) test at P = 0.05 probability. A correlation was established among fly ovipositional preference data and physiochemical characters of fruits.

### Results

#### Host preference

#### Choice test

Results regarding the oppositional response or host preference under choice conditions showed that B. *dorsalis* prefered laying eggs on fruits of C. *reticulata* as showed by maximum pupal recovery (10.67 pupae/fruit), followed by *C*. sinensis (7.50 pupae/fruit) (F = 52.7, P < 0.0001) (Fig.1a). In case of pupal weight, C. reticulata showed maximum pupal weight (9.29 mg) in comparison to other cultivars (F = 14.7, P < 0.0001) (Fig.1b). Further, significantly elevated levels of adult emergence were observed in C. aurantium (86.25%) and C. sinensis (82.26%) (F = 0.92, P = 0.04) (Fig.1c). The maximum deformity was observed in C. aurantium (43.35%), followed by C. paradisi (34.05%), which was significantly higher as compared to other cultivars (F = 2.14, P = 0.02) (Fig. 1d). In case of fly sex ratio, the highest male percentage was observed in C. sinensis (50.28 %), while the highest population of females was shown by C. reticulata (61.78%) (Table 1).

**Table 1.** The sex ratio of adult flies resulted from the fruits of citrus cultivars infested by *Bactrocera dorsalis* under choice and no-choice tests.

Citrus cultivars	Sex ratio								
	Choic	e test	No choice test						
-	Male (%)	Female (%)	Male (%)	Female (%)					
Citrus reticulata	$38.22 \pm 2.83^{ab}$	61.78±7.98 <sup>a</sup>	$37.41 \pm 2.24^{ab}$	62.59±2.24ª					
Citrus sinensis	$50.28 \pm 6.87^{a}$	49.72±6.87 <sup>ab</sup>	$49.19 \pm 7.25^{a}$	$50.81 \pm 7.25^{ab}$					
Citrus aurantium	$37.50 \pm 4.17^{ab}$	$37.50 \pm 4.17^{bc}$	$46.53 \pm 2.08^{ab}$	53.47±2.08ª					
Citrus paradisi	$31.94 \pm 6.94^{b}$	$59.72 \pm 9.72^{a}$	$30.56 \pm 6.99^{ab}$	61.11±8.14 <sup>a</sup>					
Citrus aurantifolia	$37.50 \pm 7.18^{ab}$	20.83±2.83 <sup>c</sup>	26.39±4.87 <sup>b</sup>	31.94±5.73 <sup>b</sup>					
F-value	0.55	6.27	1.93	3.80					
P-value	0.03	0.003	0.02	0.03					
LSD-value	14.49	20.47	21.38	18.95					

\*Means followed by same letters are not statistically different; LSD, (P=0.05).

### No-choice test

The results regarding host preference under the nochoice test with each citrus cultivar showed the highest pupal recovery in *C. reticulata* (8.58 pupae/fruit), while *C. aurantifolia* had least pupal recovery (2.17 pupae/fruit) (F = 44.4, P < 0.0001) (Fig. 2a). Generally, pupal weight varied less significantly among citrus cultivars with *C. reticulata* showing the highest pupal weight (8.64 mg) in comparison to other citrus cultivars (F = 13.7, P = 0.0001) (Fig. 2b). Significantly, higher adult (96.81%) observed in C. emergence was reticulata, followed by C. sinensis (88.56%) (F = 4.11, P = 0.02) (Fig. 2c). Maximum deformed emerged flies (31.94%) were observed in C. paradisi, followed by C. aurantifolia (23.61%) (F = 1.21, P = 0.03) (Fig. 2d). In case of adult sex ratio, the highest population percentage of males was observed in C. sinensis (49.19%), while C. reticulata showed the highest population of females (62.59%) (Table 1).

Table 2. Physicochemical	properties of fruits of differen	t citrus cultivars.
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Citrus cultivars		Physical parameter	er	Chemical parameter			
	Peel thickness	Fruit diameter	Fruits weight (g)	TSS (%)	Acidity (%)	TSS/acid ratio	
Citrus reticulata	0.46±0.10 <sup>c</sup>	$6.46 \pm 1.31^{bc}$	$121.5 \pm 2.54^{ab}$	$12.18 \pm 0.274^{ab}$	0.702±0.026ª	$17.58 \pm 1.042^{ab}$	
Citrus sinensis	$0.57 \pm 0.08^{b}$	6.13±1.45°	$116.1 \pm 3.21^{b}$	$11.73 \pm 0.284^{b}$	0.682±0.062ª	$16.54 \pm 0.834^{b}$	
Citrus aurantium	$0.58 \pm 0.09^{b}$	7.02±1.03ª	$118.3 \pm 1.34^{ab}$	$11.67 \pm 0.385^{b}$	$0.655 \pm 0.027^{a}$	$17.61 \pm 0.874^{ab}$	
Citrus paradisi	0.43±0.10°	$6.72 \pm 0.45^{ab}$	$124.5 \pm 2.11^{a}$	13.96±0.542ª	$0.722 \pm 0.037^{a}$	18.42±1.024 <sup>a</sup>	
Citrus aurantifolia	$0.74 \pm 0.21^{a}$	$5.44 \pm 1.21^{d}$	$103.2 \pm 3.11^{\circ}$	$9.16 \pm 0.285^{\circ}$	$0.660 \pm 0.027^{a}$	14.52±0.643°	
F-value	18.0	23.6	9.91	8.20	1.60	5.82	
P-value	0.0001	0.0001	0.0004	0.0010	0.2247	0.0049	

\*Means sharing similar letters within the column are not statistically different; LSD, (P>0.05).

## Physiochemical parameters

The data regarding physical parameters including peel thickness (F = 18.0, P < 0.001) fruit diameter (F = 23.6, P < 0.001) and fruits weight (g) (F = 9.91, P < 0.001) showed a significant difference among different citrus cultivars. The peel thickness of *C. aurantifolia* was found higher (0.74inch), followed by *C. aurantium* (0.58inch). In comparison, the peel thickness of *C. paradisi* was the lowest (0.43inch). Fruit diameter was highest in *C. aurantium* (7.02 mm), while *C. aurantii folia* (5.44mm) showed

the least fruit diameter. C. paradisi's fruit weight was (124.50 g), followed by *C. reticulata* (121.50 g).

The lowest fruit weight (103.21 g) was recorded in the case of *C. aurantifolia*. In the case of the chemical parameters, a significant difference of total soluble solids (TSS) (F = 8.20, P = 0.001) and TSS/acid ration (F = 5.82, P < 0.05) was found among different citrus cultivars. However, no significant difference (F= 1.60, P > 0.05) of acidity percentage was found among different citrus cultivars.

**Table 3.** Correlations analysis of physicochemical characters of fruits of different citrus cultivars with ovipositional preferences of *Bactrocera dorsalis* under choice test.

	Choice test									
Physiochemical	Recover	Recovered Pupae		Pupal weight (mg)		Deformity		nergence		
characters	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value		
Peel thickness	0.76	0.0001	0.63	0.0027	-0.32	0.1680	0.18	0.4375		
Fruit diameter	-0.79	0.0000	-0.31	0.1722	0.67	0.0012	0.16	0.4955		
Fruits weight (g)	-0.75	0.0001	-0.44	0.068	0.29	0.2072	-0.15	0.5059		
Total SS (%)	-0.63	0.0027	-0.36	0.1170	0.08	0.7373	-0.14	0.5316		
Acidity (%)	-0.34	0.1372	-0.39	0.0841	-0.05	0.8041	-0.28	0.2226		
TSS/acid ratio	-0.67	0.0011	-0.37	0.1051	0.27	0.2454	-0.09	0.7017		

r = correlation coeffeicient, P < 0.05 shows the significance, P > 0.05 shows the non-significance.

The percentage of TSS was higher (13.9%) in *C. paradisi*, followed by 12.18% in *C. reticulata*. The lowest percentage of 9.16% of TSS was found in *C. aurantifolia* (Table 2). The correlations analysis of physiochemical characteristics of a citrus cultivar with ovipositional preferences of *B. dorsalis* in the choice test showed that the peel thickness of fruits has a significant and positive correlation (r = 0.76) with recovered pupae. However, fruit diameter (r = -0.79),

fruit weight (r = -0.75), TSS/acid ratio (r = -0.67) and TSS (r = -0.63) showed significant (P < 0.05) and negative relation with recovered pupae. Acidity showed no significant relation with recovered pupae.

Only peel thickness showed significant and positive (r = 0.63, P < 0.05) relation with pupal weight. In case of deformity, only fruit diameter showed significant and positive (r = 0.67, P < 0.05) relation.

**Table 4.** Correlations analysis of physicochemical characters of fruits of different citrus cultivars with ovipositional preferences of *Bactrocera dorsalis* under no-choice test.

No-Choice test									
Physiochemical characters	Recovered Pupae		Pupal weight (mg)		Deformity		Adult emergence		
	R-value	P-value	R-value	P-value	R-value	P-value	R-value	P-value	
Peel thickness	0.68	0.0009	0.55	0.0107	-0.45	0.0417	0.50	0.0232	
Fruit diameter	-0.40	0.0746	-0.27	0.2389	0.33	0.1429	-0.28	0.2245	
Fruits weight (g)	-0.54	0.0120	-0.38	0.0913	0.18	0.4326	-0.47	0.0359	
Total SS (%)	-0.59	0.0055	-0.24	0.3047	0.07	0.7540	-0.36	0.1173	
Acidity (%)	-0.43	0.0536	-0.24	0.2987	0.18	0.4353	-0.36	0.1126	
TSS/acid ratio	-0.57	0.0085	-0.32	0.1682	0.15	0.5073	-0.30	0.1978	

r = correlation coefficient, P < 0.05 shows the significance, P > 0.05 shows the non-significance.

All the physicochemical parameters showed no significant (P > 0.05) relation with adult emergence (Table 3). In the case of the no-choice test, the correlations analysis of physiochemical characters of a citrus cultivar with ovipositional preferences of *B. dorsalis* showed that peel thickness of fruits has a significant and positive correlation (r = 0. 68, P < 0.001) with recovered pupae. However, fruit weight (r = -0.54), TSS/acid ratio (r = -0.59) and TSS (r = -0.57) showed significant (P < 0.05) and negative

relation with recovered pupae. Acidity and fruit diameter showed no significant relation with recovered pupae. Only peel thickness showed significant and positive (r = 0.55, P < 0.05) with pupal weight and negative relation (r = -0.45, P < 0.05) with deformity. Peel thickness also showed significant (P < 0.05) and positive (r = 0.50) relation with adult emergence. All the physicochemical parameters showed no significant (P > 0.05) relation with adult emergence (Table 4).

**Table 5.** Regression analysis of physicochemical characters of fruits of different citrus cultivars with ovipositional preferences of *Bactrocera dorsalis* under choice test.

			Choice test				
Recovered pupae		Pupal weight (mg)		Deformity		Adult emergence	
R <sup>2</sup> (Adj. R <sup>2</sup> )	Regression equation	$\mathbb{R}^2$	Regression equation	R <sup>2</sup>	Regression equation	$\mathbb{R}^2$	Regression equation
58.5 (56.2)	-6.1+20.8X1	40.2 (36.9)	0.49+11.7X1	10.3 (5.3)	52.2-51.1X1	3.4 (0.1)	40.5+47.7X1
62.8 (60.7)	34.1-4.50X1	10.1 (5.10)	14.8-1.23X1	44.9 (41.8)	-17.8+22.3X1	2.6 (0.1)	11.2+8.77X1
56.8 (54.4)	40.03-0.29X1	20.2 (15.7)	21.1-0.12X1	8.7 (3.6)	-55.4+0.67X1	2.5 (0.1)	136.0-0.59X1
40.1 (36.7)	18.6-1.13X1	13.1 (8.26)	12.2-0.44X1	0.6 (0.1)	14.1+0.838X1	6.7 (0.3)	96.6-2.53X1
11.8 (6.95)	22.2-24.5X1	15.6 (10.9)	20.1-19.2X1	0.5 (0.1)	40.8-24.7X1	8.1 (3.0)	199.6-193.6X1
45.7 (42.7)	27.4-1.302X1	13.9 (9.15)	15.3-0.48X1	7.4 (2.3)	-28.1+3.17X1	5.4 (2.3)	95.2-1.67X1
	R <sup>2</sup> (Adj. R <sup>2</sup> ) 58.5 (56.2) 62.8 (60.7) 56.8 (54.4) 40.1 (36.7) 11.8 (6.95) 45.7 (42.7)	Regression           R2 (Adj. R2)         Regression           equation         equation           58.5 (56.2)         -6.1+20.8X1           62.8 (60.7)         34.1-4.50X1           56.8 (54.4)         40.03-0.29X1           40.1 (36.7)         18.6-1.13X1           11.8 (6.95)         22.2-24.5X1	Regression equation         R <sup>2</sup> 58.5 (56.2)         -6.1+20.8X1         40.2 (36.9)           62.8 (60.7)         34.1-4.50X1         10.1 (5.10)           56.8 (54.4)         40.03-0.29X1         20.2 (15.7)           40.1 (36.7)         18.6-1.13X1         13.1 (8.26)           11.8 (6.95)         22.2-24.5X1         15.6 (10.9)           45.7 (42.7)         27.4-1.302X1         13.9 (9.15)	Regression equation         R <sup>2</sup> Regression equation         Regression equation           58.5 (56.2)         -6.1+20.8X1         40.2 (36.9)         0.49+11.7X1           62.8 (60.7)         34.1-4.50X1         10.1 (5.10)         14.8-1.23X1           56.8 (54.4)         40.03-0.29X1         20.2 (15.7)         21.1-0.12X1           40.1 (36.7)         18.6-1.13X1         13.1 (8.26)         12.2-0.44X1           11.8 (6.95)         22.2-24.5X1         15.6 (10.9)         20.1-19.2X1           45.7 (42.7)         27.4-1.302X1         13.9 (9.15)         15.3-0.48X1	$ \begin{array}{c} \mbox{R}^2  (\mbox{Adj. R}^2) \\ \mbox{R}^2  (\mbox{Adj. R}^2) \\ \mbox{equation} \\ \mbox{equation} \\ \mbox{equation} \\ \mbox{R}^2 \\ \mbox{equation} \\ \mbox{equation}$	Regression equation         Regression equation         Regression equation         Regression equation         Regression equation           58.5 (56.2)         -6.1+20.8X1         40.2 (36.9)         0.49+11.7X1         10.3 (5.3)         52.2-51.1X1           62.8 (60.7)         34.1-4.50X1         10.1 (5.10)         14.8-1.23X1         44.9 (41.8)         -17.8+22.3X1           56.8 (54.4)         40.03-0.29X1         20.2 (15.7)         21.1-0.12X1         8.7 (3.6)         -55.4+0.67X1           40.1 (36.7)         18.6-1.13X1         13.1 (8.26)         12.2-0.44X1         0.6 (0.1)         14.1+0.838X1           11.8 (6.95)         22.2-24.5X1         15.6 (10.9)         20.1-19.2X1         0.5 (0.1)         40.8-24.7X1           45.7 (42.7)         27.4-1.302X1         13.9 (9.15)         15.3-0.48X1         7.4 (2.3)         -28.1+3.17X1	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $

 $R^2$  = coefficient of determination.

In the case of the choice test, the regression analysis showed that peel thickness (adj.  $R_2 = 56.2$ ) and fruit diameter (adj.  $R_2 = 60.7$ ) showed a strong relation with recovered pupae. However, no relation was found among all the physicochemical characters of citrus cultivars with pupal weight, deformity, and adult emergence (Table 5). However, in the no-choice test, all the physiochemical characters showed a weak relation with all parameters of the ovipositional preference of *B. dorsalis* (Table 6).

**Table 6.** Regression analysis of physiochemical characters of fruits of different citrus cultivars with ovipositional preferences of *Bactrocera dorsalis* under no-choice test.

		No-cł	noice test				
Recovered Pupae		Pupal weight (mg)		Deformity		Adult emergence	
R <sup>2</sup> (Adj. R <sup>2</sup> )	Regression equation	R <sup>2</sup>	Regression equation	R <sup>2</sup>	Regression equation	R <sup>2</sup>	Regression equation
46.6 (43.6)	-2.01+15.2X1	31.0 (27.2)	1.59+9.58X1	21.1 (16.7)	53.9-66.4X1	25.5 (21.3)	9.1+116X1
16.6 (12.1)	18.5-1.90X1	7.6 (2.5)	13.2-0.99X1	11.5 (6.6)	-48.1+10.3X1	8.1 (3.1)	160-13.7X1
30.2 (26.4)	27.1-0.177X1	15.0 (10.3)	18.1-0.09X1	3.9 (0.9)	-28.3+0.38X1	22.2 (17.9)	257-1.57X1
35.6 (32.1)	16.7-0.87X1	5.8 (0.6)	10.1-0.27X1	3.4 (0.6)	8.7+0.71X1	13.1 (8.2)	138-5.47X1
19.2 (14.7)	23.9-25.1X1	6.3 (2.1)	14.4-11.1X1	3.4 (0.4)	-30.9+70.1X1	13.4 (8.6)	225-221X1
32.7 (28.9)	21.7-0.91X1	10.3 (5.3)	13.5-0.38X1	2.9 (0.5)	-10.1+1.61X1	9.3 (4.2)	156-4.89X1
	R <sup>2</sup> (Adj. R <sup>2</sup> ) 46.6 (43.6) 16.6 (12.1) 30.2 (26.4) 35.6 (32.1) 19.2 (14.7)	R2 (Adj. R2)         Regression equation           46.6 (43.6)         -2.01+15.2X1           16.6 (12.1)         18.5-1.90X1           30.2 (26.4)         27.1-0.177X1           35.6 (32.1)         16.7-0.87X1           19.2 (14.7)         23.9-25.1X1	Recovered Pupae         Pupal w           R² (Adj. R²)         Regression equation         R²           46.6 (43.6)         -2.01+15.2X1         31.0 (27.2)           16.6 (12.1)         18.5-1.90X1         7.6 (2.5)           30.2 (26.4)         27.1-0.177X1         15.0 (10.3)           35.6 (32.1)         16.7-0.87X1         5.8 (0.6)           19.2 (14.7)         23.9-25.1X1         6.3 (2.1)	R2 (Adj. R <sup>2</sup> )         Regression equation         R <sup>2</sup> Regression equation           46.6 (43.6)         -2.01+15.2X1         31.0 (27.2)         1.59+9.58X1           16.6 (12.1)         18.5-1.90X1         7.6 (2.5)         13.2-0.99X1           30.2 (26.4)         27.1-0.177X1         15.0 (10.3)         18.1-0.09X1           35.6 (32.1)         16.7-0.87X1         5.8 (0.6)         10.1-0.27X1           19.2 (14.7)         23.9-25.1X1         6.3 (2.1)         14.4-11.1X1	Recovered Pupae         Pupal weight (mg)         Def           R² (Adj. R²)         Regression equation         R²         Regression equation         R²           46.6 (43.6)         -2.01+15.2X1         31.0 (27.2)         1.59+9.58X1         21.1 (16.7)           16.6 (12.1)         18.5-1.90X1         7.6 (2.5)         13.2-0.99X1         11.5 (6.6)           30.2 (26.4)         27.1-0.177X1         15.0 (10.3)         18.1-0.09X1         3.9 (0.9)           35.6 (32.1)         16.7-0.87X1         5.8 (0.6)         10.1-0.27X1         3.4 (0.6)           19.2 (14.7)         23.9-25.1X1         6.3 (2.1)         14.4-11.1X1         3.4 (0.4)	Recovered Pupae         Pupal weight (mg)         Deformity           R² (Adj. R²)         Regression equation         R²         Regression equation         R²         Regression equation           46.6 (43.6)         -2.01+15.2X1         31.0 (27.2)         1.59+9.58X1         21.1 (16.7)         53.9-66.4X1           16.6 (12.1)         18.5-1.90X1         7.6 (2.5)         13.2-0.99X1         11.5 (6.6)         -48.1+10.3X1           30.2 (26.4)         27.1-0.177X1         15.0 (10.3)         18.1-0.09X1         3.9 (0.9)         -28.3+0.38X1           35.6 (32.1)         16.7-0.87X1         5.8 (0.6)         10.1-0.27X1         3.4 (0.6)         8.7+0.71X1           19.2 (14.7)         23.9-25.1X1         6.3 (2.1)         14.4-11.1X1         3.4 (0.4)         -30.9+70.1X1	Recovered Pupae         Pupal weight (mg)         Deformity         Adult en           R² (Adj, R²)         Regression equation         R²         Regression equation         R²         Regression equation         R²           46.6 (43.6)         -2.01+15.2X1         31.0 (27.2)         1.59+9.58X1         21.1 (16.7)         53.9-66.4X1         25.5 (21.3)           16.6 (12.1)         18.5-1.90X1         7.6 (2.5)         13.2-0.99X1         11.5 (6.6)         -48.1+10.3X1         8.1 (3.1)           30.2 (26.4)         27.1-0.177X1         15.0 (10.3)         18.1-0.09X1         3.9 (0.9)         -28.3+0.38X1         22.2 (17.9)           35.6 (32.1)         16.7-0.87X1         5.8 (0.6)         10.1-0.27X1         3.4 (0.6)         8.7+0.71X1         13.1 (8.2)           19.2 (14.7)         23.9-25.1X1         6.3 (2.1)         14.4-11.1X1         3.4 (0.4)         -30.9+70.1X1         13.4 (8.6)

 $R^2$  = coefficient of determination.

## Discussion

The ovipositional preference and selection of proper host in fruit flies is imperative as proper host selection supports the development parameters of their progeny (Papaj and Aluja, 2008; Kachigamba *et*  *al.*, 2012; Silva *et al.*, 2012). The current study aimed to evaluate the ovipositional preferences of *B. dorsalis* in different citrus cultivars under laboratory conditions.



**Fig. 1.** Number of recovered pupae per fruit (a), pupal weight (mg) (b), adult emergence (%) (c) and deformity (d) of *Bactrocera dorsalis* on fruits of different citrus cultivars under choice test. Means followed by the same letters are not statistically different (onw-way ANOVA; LSD at P=0.05).

In the present study, B. dorsalis showed maximum infestation on C. reticulata, followed by C. sinensis in no choice and choice tests compared to other cultivars. An infestation of Bactrocera invadens Drew, Tsuruta, and White (Diptera: Tephritidae) has been reported on C. sinensis and C. reticulata in Africa supporting the findings of the current study (Rwomushana et al., 2008; Danjuma et al., 2014). Allwood et al. (1999) and Clarke et al. (2005) also confirmed the results of our study as B. dorsalis infests citrus cultivar. As predicted, females should allocate their progeny in habitats to make the most available resources (Nufio and Papaj, 2004). Significant influences of host preference on progeny performance have been documented previously (Alistair and Steinberg, 1999; Harvey et al., 2014).In

our study, the difference in the recovered pupae per fruit laid in each citrus cultivar clearly showed that *C. aurantifolia* was not equally attractive to *B. dorsalis* at both no choice and free condition. Spitler *et al.* (1984) observed similar results where most lemon cultivars appeared to be almost immune to the attack of *Ceratitis capitata* (Wiedemann). Besides, Papanastasiou *et al.* (2020), Papanastasiou *et al.* (2017) and Katsoyannos *et al.* (1999) reported that the lemon cultivar was least attractive to flies attack.

Further, Staub *et al.* (2008) reported mandarins and oranges are suitable for the development of *C.capitata*, while lemons are poor hosts regardless of conditions provided to support the findings of the present study.



**Fig. 2.** Number of recovered pupae per fruit (a), pupal weight (mg) (b), adult emergence (%) (c) and deformity (d) of *Bactrocera dorsalis* on fruits of different citrus cultivars under no-choice test. Means followed by the same letters are not statistically different (onw-way ANOVA; LSD at P=0.05).

Other fruit fly activities, especially adult emergence and deformity in both no choice and free condition, showed that *C. aurantifolia* and *C. paradisi* showed significant cultivar. Previous studies showed that sweet cultivars of some fruit showed more infestation while sour cultivars of the same fruit had less or no fruit fly infestation (Staub *et al.*, 2008). Further, the oviposition preference of fruit flies depends on other factors, including smell and visual signs, for identifying potential ovipositional sites (Zhang *et al.*, 2017). In some cases, fruit flies use prior experience to find and select a host fruit for oviposition

(Papachristos *et al.*, 2008; Rattanapun *et al.*, 2009) and females show learning ability in the selection of suitable host (Raga *et al.*, 2004). In the case of nochoice, as explained previously by Díaz-Fleischer *et al.* (2014), fruit flies always have a distinct liking for those fruits that came in contact with and were visited earlier by other female fruit flies. Further, for choosing one host over another, fruit flies' preference for the original host remains foremost as certain fruit characters, i.e., the nutritional value of fruit, have a vital role in larval development.

## Conclusion

Our study demonstrated that *C. aurantifolia* and *C.* paradisi are poor hosts, and both hosts not only affect oviposition preference but also influence the progeny by showing consequences on adult emergence and deformity. То determine limits of Bdorsalis ecological range, knowledge regarding the intrinsic rate of B. dorsalis concerning natural limiting factors may aid in controlling its spread. Citrus fruits' physiochemical characteristics are significantly different across the citrus cultivars; however, there was no relation of these characters on the ovipositional behaviour of B. dorsalis. Only the peel thickness, fruit weight, and diameter have a limited role in ovipositional behaviour. Our results indicate that irrespective of the female preference for eggs lying on citrus fruits, females can attack bunches of all citrus cultivars, although the citrus fruit has adverse physiochemical properties egg-laying of B. dorsalis. A deeper understanding of the relationships for both 'fruit chemistry' or the conduct of oviposition of fruit flies delivers a dynamic aspect for the adoption of sound farming practices aimed at preventing or reducing crop damage.

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