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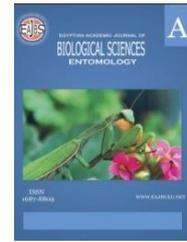
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Oviposition Preference and Performance of *Bactrocera dorsalis* Hendel, (Diptera: Tephritidae) on Four Colour Types of Willard mango (*Mangifera indica* L)

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ABSTRACT

Bactrocera dorsalis Hendel is the most widespread serious fruit pest in Asia and the knowledge on oviposition behaviour of *B. dorsalis* on different fruit varieties are vital for effective management of the pest. Hence, the present study investigated the preference and oviposition behaviour of *B. dorsalis* on different colour types of Willard mango variety. A series of choice and non-choice laboratory experiments for testing the oviposition preference were conducted and tested fruits were incubated until pupation and the emergence of adults. The results revealed that female *B. dorsalis* showed a significantly high preference to visit and oviposit in yellow colour Willard mangoes ($p<0.05$) over red, orange and green colours. The number of pupae and number of adult flies that emerged was also significantly high in yellow colour mangoes under choice condition ($p<0.05$). In both choice and non-choice tests, green colour Willard was less prominently used by female flies for their visiting and ovipositing ($p<0.05$). The number of visits and visit duration of fruits was positively correlated with oviposition attempts of female flies in choice conditions. In both tests, the number of oviposition attempts was significantly correlated with the number of pupae and adults who emerged. There was no influence of the colour on the percentage of adult emergence from the pupae in non-choice trials. The study findings could be incorporated to plan and implement the control measures to avoid infestations of commercial mango varieties by fruit flies at the field and at the market level.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are common agricultural pests, they damage fruits and vegetables making a significant economic loss. Most of the species are polyphagous and they survive on a wide range of host plants. Female fruit flies deposit eggs inside the host fruits or vegetables using their ovipositor. The flesh inside of mature fruits is subsequently consumed by their larvae and before pupation, mature larvae moved outside and usually pupate in the soil. When developing larvae, they feed inside the host fruits and thus fruits become unsuitable for consumption (Mohd *et al.*, 2011). The development of larvae of fruit flies depends on the quality of available nutrients of the

host fruit, fruit size, development period, and maturation time of adult flies (Krainacker *et al.*, 1987; Bruzzone *et al.*, 1990; Hing, 1991; Khan *et al.*, 1999; Kaspi *et al.*, 2002).

Fruit flies can select fruits that are suitable for their oviposition and the survival of their larvae (Fitt, 1981; Joachim-Bravo *et al.*, 2001; Fontellas-Brandalha & Zucoloto, 2004). The affinity of female flies is influenced by colour, size, shape, and smell of fruit (Prokopy & Owens, 1983; Jang & Light, 1991; Prokopy & Vargas, 1991; Cornelius *et al.*, 1999; Alyokhin *et al.*, 2000; Drew *et al.*, 2003; Brevault & Quilici, 2007) and the ripens stage of fruits (Rattanapun *et al.*, 2009). Most fruit fly species in dacine group prefer to oviposit in both ripe and over-ripe fruits (Fletcher, 1987; Allwood, 1997). Drew *et al.* (1978) and Dorji *et al.* (2006) recorded *Bactrocera musae* (Tryon) and *Bactrocera minax* (Enderlein) damage to immature fruits. However, few past studies were done to investigate the oviposition behaviour of fruit flies on different physical characteristics of fruits (Rattanapun *et al.*, 2009, Vignesh & Chandrasekaran, 2020). Several other studies have been reported the visual responses of *B. dorsalis* were high in yellow colour artificial traps (Prokopy & Owens, 1983; Vargas *et al.*, 1991; Cornelius *et al.*, 1999). In addition, the colour preference for the oviposition of *B. zonata* was studied by Nusrat *et al.* (2019) and they revealed *B. zonata* most preferred to oviposit in a sky-blue colour artificial surface which smeared with mango paste. In 2017, Zaheer *et al.*, reported fruit flies mostly attract yellow and transparent coloured traps. Similarly, Kavita & Sandeep (2020) recorded males of *Bactrocera* spp. are highly attracted to yellow-coloured methyl eugenol traps. However, there are no sufficient previous studies reported on the colour preference of fruit flies for oviposition using natural fruits.

Bactrocera dorsalis, the oriental fruit fly, is one of the severe fruit pest species of the genus *Bactrocera* Macquart which has more than 440 described species in the tropics (Leblanc, 2013) including Asia, the South Pacific, and Australia (Vargas *et al.*, 2015). The *B. dorsalis* maintain high pest status than other species of the group (Drew and Hancock, 1994) because they are highly polyphagous, have high reproductive potential, continuous generations throughout the year and high flight range (Leblanc, 2013). This species is most virulent on different commercial fruits, causing high damage to mango (*Mangifera indica* L.) (Clarke *et al.*, 2005). Previous studies in Sri Lanka have been intended mainly on control strategies of *B. dorsalis* (Anonymous, 2012; Dhanapala, 1996; Karunarathna, 2012) rather than their economic damages. Studies of the oviposition behavior of *B. dorsalis* on commercial host fruits are obviously lacking in Asia and Sri Lanka as well.

A wide range of mango varieties (*Mangifera indica* L.) is grown in Sri Lanka. Among them, “Karutha kollomban”, “Willard” and “Velleikollomban” varieties have high commercial value. In 1996, Dhanapala reported *B. dorsalis* is caused severe yield loss in mango among other fruit crops in Sri Lanka and their damage is more than 50% in mango varieties of 'Karutha Kolamban', 'Wellai Kolamban' and 'Willard'. The present study was therefore conducted to determine the performance and preference for the oviposition of *B. dorsalis* in choice and non-choice tests using four colours of ripe mango of Willard variety under controlled laboratory conditions.

Since studies on the susceptibility of various colours of commercial mango to *B. dorsalis* in Sri Lanka are lacking and this information is important for farmers to take precautions to avoid the infestation of particular colour fruit by *B. dorsalis*. Hence, the study information will be useful to maximize the profit of mango cultivators.

MATERIALS AND METHODS

Culture of *B. dorsalis*:

The rearing of *B. dorsalis* was maintained in the research laboratory of the Department of Zoology, University of Ruhuna, Sri Lanka. Randomly selected over-ripe and ripe mangoes were collected from the ground and from the trees to get fruit-fly-infested fruit specimens from two selected mango cultivated lands (Monaragala: 6°45'0"N, 81°14'0"E, elevation-162m, Wellawaya: 6° 44' 15.85" N 81° 6' 11.005" E, elevation-188m.) in Uva Province of Sri Lanka. The number of fruits being collected from each site was not pre-decided because the ultimate objective was to prepare a *B. dorsalis* adult culture of up to 200 individuals within the study period. Collected fruits were placed in plastic containers with pre-sterilized sand and muslin cloth cover (Temperature: 25⁰ C, RH: 75-85 %) until the emergence of adults. Emerged adult flies were transferred into plastic cages covered by muslin cloth on the top of the container. Fruit fly diet containing yeast and sugar in the ratio 1:3 + water (Ekesi and Billah, 2009) was supplied to adult flies into the cages. After seven days, both males and females of *B. dorsalis* were identified and transferred to a separate cage and kept together for breeding. The required mature females were taken out from the cages and used for both choice and non-choice laboratory tests.

Test Fruits:

Mango fruits in the Willard variety in four different colours (red, yellow, orange, and green) were selected for the study (Fig. 1). All fruits were brought from the local market at the ripened stage. Fruits only covered by the paper were bought from the market to avoid any infestations of wild flies. To check for possible field infestation of fruits, every fruit was visually examined for possible oviposition marks of fruit flies with the aid of a hand lens. Fruits with no visual oviposition holes were used for tests. To confirm the field infestation, five fruits from each colour were randomly selected from the sample and incubated individually in plastic see if any pupae or adults were recovered.



Fig. 1: Willard mango in red, yellow, orange and green colours

Testing Containers:

The standard size plastic containers with 18 ×14 ×13 cm in length, width, and height respectively were used for the test. Each container was lined with pre-sterilized sieved sand in 6 cm height. Muslin cloth in 1mm mesh size was used to tightly cover the top of the container. Elastic rubber bands were used to tighten the muslin cover to prevent flies from entering or escaping the rearing container. Containers were placed in water baths to protect experimental setups from ants. Testing containers were stored under Temperature: 25⁰ C, RH: 75-85 % in the laboratory.

Choice Test:

Four mangoes of Willard variety in each red, yellow, orange, and green colour were chosen as replicates. Totally, eighty fruits for twenty replicates were used. All fruits were weighed and labelled. Four fruits of four colours and approximately of the same weight ($\pm 5.00\text{g}$) were kept on the sand layer inside the testing container keeping the same gap among each fruit (Fig. 2). A mature female (8-15 days old) which has obtained from the culture was released into the centre of a testing container. The observed and recorded behaviours of fruit flies for 3 hrs (10.00- 13.00 hrs) were: (a) choice for the visit of each fruit (b) number of visits of each fruit, (c) Number of oviposition attempts in each fruit, (d) visit duration in each fruit. Twenty single fly replicates were conducted using eighty fruits and twenty testing containers following the same method. After three hours, the tested fly was removed from the container. Then each fruit was visually examined for oviposition marks.

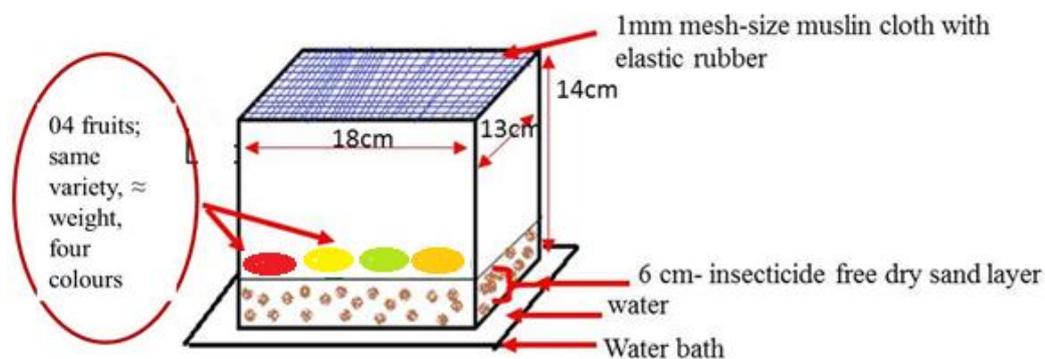


Fig. 02: Experimental setting in the choice test for oviposition of *B. dorsalis* on different colours of Willard mangoes

Non-choice test: Willard fruits in each colour were placed individually in testing containers (Fig. 3). The oviposition behaviour was observed inserting a mature female into the container for 3 hrs. period. All other steps and experimental conditions were followed for the choice test except the simultaneous offering of fruits of four colours.

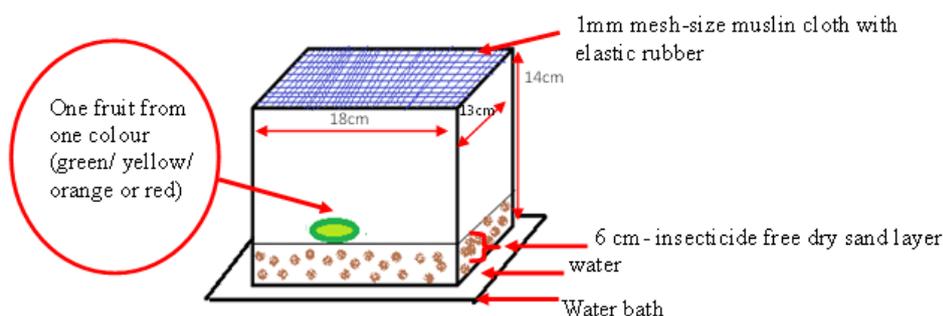


Fig. 3: Experimental setting in the non-choice test for oviposition of *B. dorsalis* on different colours of Willard mangoes

The Emergence of Pupae and Adults:

All tested fruits in choice and non-choice experiments were carefully examined for oviposition marks. Then all fruits were labelled and incubated individually in plastic containers with pre-sterilized sand and muslin cloth cover under controlled laboratory conditions (Temperature: 25°C , RH: 75-85 %). At the end of the fourth week, the

containers were carefully examined and all pupae and adult flies (males and female) were counted.

Statistical Analysis:

Data were coded and entered into a database created using the Statistical Package for the Social Sciences (SPSS) software. Data checking and cleaning were done. The data set was tested to the normality using the software. The significance in the variations of the number of visits, visit duration, the number of oviposition attempts by female flies, and the number of pupae and adults emerged per colour of mango in each choice and non-choice conditions were compared using one-way ANOVA analysis at $\alpha=0.05$ significance level. Relationships between the number of oviposition attempts with the number of visits, visit duration and number of pupae and adults emergence per colour of mango in each choice and non-choice conditions were examined by Pearson's correlation analysis at $\alpha=0.01$ significance level.

The ethical approval for the study was obtained by the Ethical Review Committee, Faculty of Science, University of Kelaniya, Sri Lanka (UOK/ERC/FS/21/023).

RESULTS AND DISCUSSION

Willard is a medium-size mango variety compared to the other mango varieties. Red, yellow, green, and orange colour ripe mangoes of the Willard variety are common at the market.

Choice test

The mean weight (g) (mean \pm SE) of Willard mango selected for the study was (112.88 \pm 1.05) g. The preference for colour by female *B. dorsalis* for 1-10 occasions is illustrated in figure 4. Flies selected yellow as the most preferred colour on nine occasions out of the ten. The selection of red colour was moderate in choice 1-10 except the choice 3. The flies showed less preference to visit green and orange colour mangoes during ten occasions.

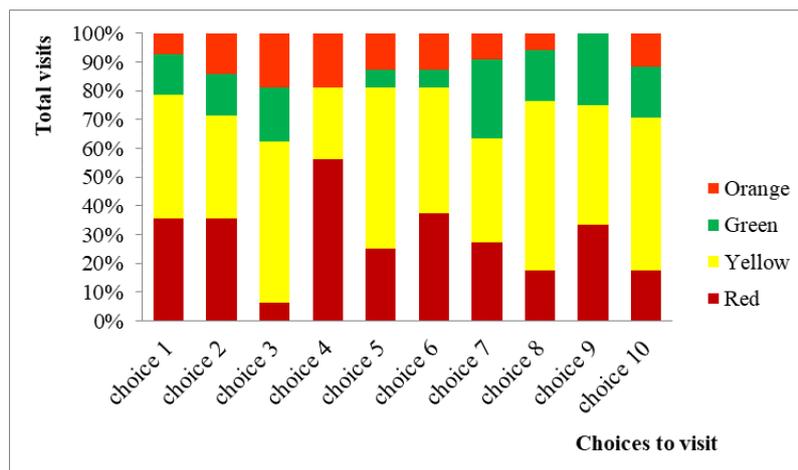


Fig. 04: Total visits of *B. dorsalis* for different colour mangoes in choice tests.

Means of visits, oviposition attempts, and visit duration of *B. dorsalis* were determined on a per colour basis and illustrated in Figure 05 (1, 2, and 3). The number of visits varies significantly among the colour of the Willard mango (ANOVA; $p= 0.00$). The highest number of visits was recorded for yellow Willard (ANOVA; $p= 0.00$) and, red and orange moderately visited whereas it is significantly low for green mangoes

($p < 0.05$) (Fig. 5.1).

On average, fruit colours significantly interact with the duration of visits by female flies (ANOVA; $p = 0.00$). Female *B. dorsalis* spent a longer period for visits to yellow than the other three colours ($p < 0.05$). Fruit visit duration to red colour was intermediate and, green and orange were visited significantly shorter ($p < 0.05$) when compared with yellow (Fig. 5.2).

Attempts to penetrate the skin of the Willard mango were considered as oviposition attempts by the female fly. The number of attempts for the oviposition differed significantly between the various colours of Willard mangoes (ANOVA; $p = 0.00$). Fewer oviposition attempts were observed for the green colour (Fig. 5.3). The number of oviposition attempts was significantly high for yellow ($p < 0.05$) and red ($p < 0.05$) Willard than green and orange colours. The oviposition attempts significantly and positively correlated with the number of visits ($r = 0.703$; $p = 0.000$) done by female *B. dorsalis* and with their visit duration ($r = 0.698$; $p = 0.000$).

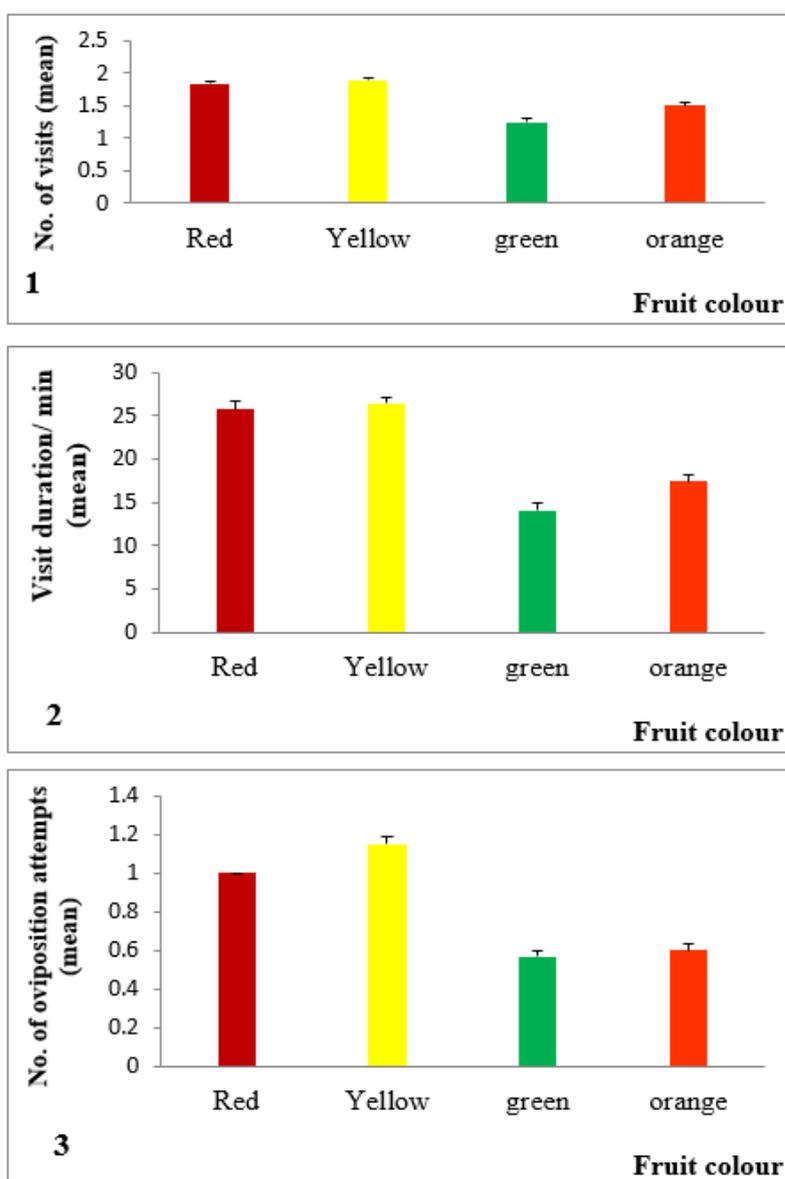


Fig. 05: Mean (+ SE), (1) No. of visits, (2) Visit duration/min and (3) No. of oviposition attempts by female *B. dorsalis* on four different colour fruits of Willard mango in choice condition.

The Emergence of Pupae and Adults:

The maximum number of pupae and emergence of adult flies was observed in yellow mangoes ($p < 0.05$) compared to the other three colours (Table 1). The lowest number of pupae and adult flies were recorded from green colour. The number of pupae (ANOVA; $p = 0.00$) and adults (ANOVA; $p = 0.00$) emerged were significantly different between the four colours. The highest percentage of adults emerged from their pupae in orange Willard and, percentages are moderate for both yellow and red colours. In fact, the overall observation of the sex ratio of adult *B. dorsalis* showed that the emergence of adult females was slightly higher when compared with the males. The number of oviposition attempts showed a significant and positive correlation with the number of pupae formed ($r = 0.584$; $p = 0.000$) and the number of adult flies ($r = 0.590$; $p = 0.000$) emerged.

Table 1: The emergence of *B. dorsalis* pupae and adults from four colour types of Willard mangoes used for oviposition preference

Colour	Mean weight of fruit (\pm SE) g	Mean No, (\pm SE) of pupae emerged	Mean No. (\pm SE) of adults emerged	Adult emergence (%)	Sex ratio M: F
Red	116.754 \pm 1.1	1.8 \pm 0.08	1.0 \pm 0.05	55.5	0.4:1.0
Yellow	115.5 \pm 1.13	5.1* \pm 0.11	3.3* \pm 0.07	64.7	0.6:1.0
Orange	111.9 \pm 0.99	0.9 \pm 0.07	0.7 \pm 0.05	77.7	0.7:1.0
Green	107.38 \pm 1.31	0.2 \pm 0.03	0.1 \pm 0.01	50.0	1.0:1.0

*The mean difference is significant at the 0.05 level

Non-Choice Test:

Ripen fruits of Willard variety (weight 115.38 \pm 2.69) g in four different colours were used for the non-choice test. In this case, the number of visits of female *B. dorsalis* was significantly differed among four colours (ANOVA; $p = 0.03$), with both yellow and red fruits had higher visits ($p > 0.05$), moderate visits to orange and lower visits to the green colour type ($p < 0.05$) (Fig. 6.1).

When considering the visit duration of *B. dorsalis* among different colours of mangoes, the highest duration was recorded for yellow ($p > 0.05$), moderate durations for red and orange and, the shorter period for green fruits ($p > 0.05$). The duration of fruit visits by female fly differed significantly between four colours (ANOVA; $p = 0.02$) (Fig. 6.2).

Female flies attempted to oviposit mostly to yellow and red colour Willard when compared to the orange and green though not significant ($p > 0.05$). The number of attempts to oviposit by females was significantly different among four colours of mangoes (ANOVA; $p = 0.04$) (Fig. 6.3). The number of oviposition attempts shows a moderately significant correlation with visit duration ($r = 0.515$; $p = 0.000$) done by female *B. dorsalis* and there was no correlation with the number of visits.

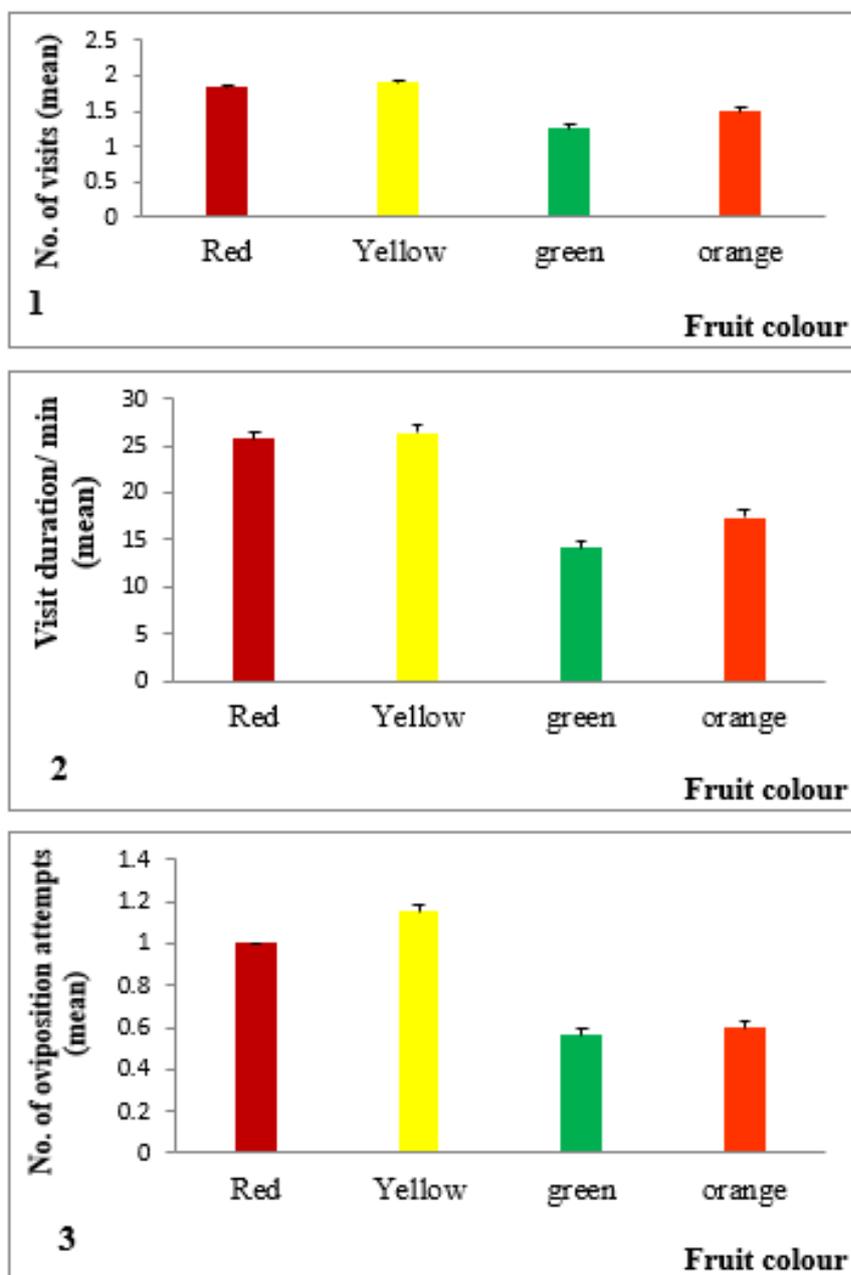


Fig. 06: Mean (+ SE), (1) No. of visits, (2) Visit duration/min and (3) No. of oviposition attempts by female *B. dorsalis* on four different colour fruits of Willard mango in non-choice condition

The Emergence of Pupae and Adults:

The results in Table 2 revealed that the emergence of pupae and adults was a little high in yellow Willard ($p > 0.05$). The lowest emergence of larvae and adults was observed from orange colour Willard. The emergence of pupae and adults was not significantly different among the four colours of Willard mango ($p > 0.05$). Interestingly, in the non-choice test, high percentages of adults have emerged from their pupae in yellow colour mangoes. The overall observation indicates that both sexes of *B. dorsalis* emerged in a 1: 1 ratio. The number of oviposition attempts showed a significant and moderate correlation with the number of pupae ($r = 0.277$; $p = 0.010$) and the number of adult flies ($r = 0.302$; $p = 0.006$) emerged.

Table 2: The emergence of pupae and adults of *B. dorsalis* from Willard mangoes at four colours in choice condition

Colour	Mean weight (\pm SE) g	Mean (\pm SE) no. of pupae emerged	Mean (\pm SE) adults emerged	Adult emergence (%)	Sex ratio M: F
Red	122.48 \pm 1.22	2.6 \pm 0.14	1.6 \pm 0.11	61.5	0.8:1.0
Yellow	116.01 \pm 1.44	3.3 \pm 0.19	2.1 \pm 0.15	63.6	0.8:1.0
Orange	99.92 \pm 0.98	1.65 \pm 0.1	0.65 \pm 0.05	39.4	1.0:0.9
Green	123.13 \pm 0.9	1.6 \pm 0.09	1.0 \pm 0.06	62.5	1.0:0.8

DISCUSSION

The overall results in this study strongly indicated that female *B. dorsalis* show a high preference to oviposit yellow colour Willard mangoes than red, orange, and green colour mangoes. These results coincide with the past findings of Zaheer *et al.*, (2017) and Kavita & Sandeep, (2020) and both studies have shown that *Bactrocera* spp. are highly attracted to yellow colour surfaces than other colours. Skin colour is one of the dependent factors to attract female flies to fruits and that was well strengthened by Aluja & Prokopy, 1993; Prokopy & Vargas, 1991; Drew *et al.*, 2003; Brevault & Quilici, 2007 showing the colour and odor of the fruit are most important factors to attract gravid female fruit flies. The findings of Prokopy & Owens, 1983; Vargas *et al.*, 1991; Cornelius *et al.*, 1999 further shown that yellow surface colour is most attractive to female *B. dorsalis* and it was in accordance with the current results. In 2001, Madura reported that the reason for the attraction of fruit flies to yellow surfaces was probably due to the reflection of yellow colour.

The high oviposition preference and offspring performance in yellow colour Willard may be explained with the characteristics of fruits. At the ripening stage of fruits, acids and starch convert to free sugars, thus fruits become to have softer pericarp (Bidwell, 1979; Yashoda *et al.*, 2007) and which provide more suitable habitat for larval survival, larval development, and shorter larval development times of fruit flies (Rattanapun *et al.*, 2009). High preference of female *B. dorsalis* to select mangoes with yellow pericarp might indicate the fruits are ripe and provide suitable conditions for higher survival and performance of larvae. In contrast, the number of visits, duration, oviposition attempts and, pupal and adult emergence of *B. dorsalis* is significantly low in green colour Willard when compared with yellow mangoes. This result is perhaps not surprising because most empirical past studies have shown that *B. dorsalis* less prefer to oviposit into unripe mangoes (Rattanapun *et al.*, 2009) because unripe fruits have high acids and low free sugars (Bidwell, 1979; Medlicott & Thompson, 1985). Thus, it is clear why female flies showed a low preference for green Willard mangoes for their oviposition even though these fruits are well rippled.

In both choice and non-choice conditions, female flies preferred to visit and attempt to oviposit moderately in both red and orange colour Willard. These results may be a link with the visual responses of *B. dorsalis*, because the studies of Vargas *et al.* (1991) and Cornelius *et al.* (1999) revealed that fruit flies captured less on orange, red, light green, dark green, blue, and black colour traps.

Our study shows that the oviposition chances of *B. dorsalis* increase with the time duration spend for fruit visits. It is well known that there is a high possibility to increase the oviposition with the long visit duration of female flies on their host fruit. Interestingly, in the case of non-choice tests, pupal and adult emergence was not significantly different among the four colours of Willard. The reason may be the gravid

females have no option to select their preferable oviposition surface colour because of the absence of simultaneous offering of different colour mangoes similar to the choice condition. Hence, they may tend to lay eggs in mangoes ignoring the host fruit colour. In this case, *B. dorsalis* showed a moderate attempt for the oviposition in green colour Willard similar to the orange colour. These results could be linked with the findings of Rattanapun *et al.*, (2009), who reported low levels of attack by *B. dorsalis* to green fruits in Asia. In 1997, Drew & Hancock reported the unusual observation of maggots of *B. dorsalis* in green papaya and Wen-Yen *et al.* (1986) recorded the attraction of *B. dorsalis* to a green colour artificial paper.

The percentage adult emergence and sex ratio of *B. dorsalis* differ with the colour of Willard in choice condition while they were not affected on the fruit colour in non-choice condition.

Indeed, the emergence of pupae and adults in high numbers from yellow Willard clearly coincides with the suitable host fruit environment for the larval performance (Diaz-Fleischer & Aluja, 2003a). It is clear, that the performance of pupae and larvae correlated with the oviposition attempts. However, controversial results have been reported by Rattanapun *et al.*, (2009) that very low oviposition rates and poor offspring survival of *B. dorsalis* at three ripening stages of mango. They tested mangoes after covering the base of the pedicel area of the fruit. They further suggested that perhaps wild flies have a high ability to oviposit into fruits over lab flies because wild flies tend to lay eggs in existing wounds and soft spots in the fruit. In the present study, we also observed that female *B. dorsalis* mostly attempted to oviposit at the base of the pedicel area of the mango. Hence it is clear why oviposition attempts of flies significantly correlate with their pupal and adult emergence in this study.

This valid information will be useful to the farmers, fruit sellers, and buyers to take precautions to avoid the infestation of fruit flies at the field, market, and at home as well. Meanwhile, the findings of the present study are very much applicable when designing and planning natural or artificial control measures against the economic loss of mango production by *B. dorsalis*.

Conclusions

The colour of Willard mangoes significantly affects the oviposition preference and the emergence of pupae and adults of *B. dorsalis*. Yellow colour Willard mangoes are more susceptible to the damage by female *B. dorsalis* for their oviposition compared to red, orange, and green mangoes. The yellow colour Willard mangoes are also best for the offspring survival with a significantly high number of pupae and adults emergence. The oviposition and adults emergence was low in green Willard. The colour choice, the number of visits, and visit duration of *B. dorsalis* positively affect their oviposition behaviour. The fruit colour influences the pupal and adult performance of *B. dorsalis*. The emergence of pupae and larvae positively influences oviposition attempts of female *B. dorsalis*. The emergence percentage of adult *B. dorsalis* does not affect the colour of mango unless simultaneous offering of various colours mangoes for the choice preference.

Conflicts of Interest: The authors would like to declare that there are no conflicts of interest in undertaking this research.

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