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Color preferences of fruit flies to methyl eugenol traps, population trend and dominance of fruit fly species in citrus orchards of Sargodha, Pakistan

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Abstract
The present study was conducted on evaluating the color preferences of fruit flies and their population dynamics in citrus orchards of Citrus Research Institute, Sargodha by installing traps of 11 different colors in three replicates. This research work was done in the year 2016 and 2017 from July to September. It was found from the pooled data of both years that maximum populations of fruit flies (irrespective of species) were attracted to yellow (78.59±16.43 flies/trap/week) and transparent (73.68±14.27 flies/trap/week) colored traps. In the year, 2016, peak population with 5151 flies was observed in the August, however in the year 2017, two peaks were observed in the 3rd week of August (1937 flies) and September (1975 flies). The species of fruit flies identified from citrus orchards were *Bactrocera zonata* and *B. dorsalis*. *B. zonata* with occurrence of 89.14%-91.66% was highly dominant in citrus orchard as compared to *B. dorsalis* with 8.33%-10.85% occurrence. From this experiment it can be concluded that fruit flies show more attraction to yellow and transparent colored traps, therefore timely installation of these traps is recommended as a part of integrated pest management for monitoring, detection and control of fruit flies.

Keywords: Fruit fly; Citrus; monitoring; Traps; Methyl eugenol; Spinosad

1. Introduction
Citrus (Sapindales: Rutaceae) is one of the widely grown fruit crops in the world with annual global production 102 million tones approximately [1] and in Pakistan area under citrus cultivation is 195,000 hectares with annual production of 1.98 million tons [2]. Pakistan ranks 12th in production of citrus in the world. Share of Punjab, Pakistan in production of citrus is 95%. Among the citrus group, Kinnow Mandarin (*Citrus reticulata* Blanco) is dominating the citrus market and is called the king of all the varieties for its best taste and flavor. Punjab produces about 70% Kinnow Mandarin which holds a key importance due to its export to other countries [3-6]. Citrus fruits are also rich source of vitamins, antioxidants, phytochemicals and have great dietary and health significance [7].

The family Tephritidae belongs to order Diptera and contains 4000 species throughout the world [8]. Out of 4000 species there are 44 species from genus *Bactrocera* which are important agricultural pests and are distributed in tropical, subtropical and temperate regions of world [9-11]. The most dominant species of fruit flies in Pakistan are *Bactrocera dorsalis*, *B. zonata* and *B. cucurbitae* out of 11 reported species [12, 13]. Fruit flies are polyphagous pests and cause losses both quantitatively and qualitatively to wide range of vegetables and fruits over the world [14]. Many countries which import the vegetables and fruits have made it compulsory to treat the commodity before their importation to kill the fruit fly larvae and also imposed the ban on the import of commodities infested with fruit flies because fruit flies are potential invasive pests [15].

Many member of Tephritidae are specialized having few host range and some are generalist that feed on many vegetables and fruits [10]. Among the members of Tephritidae, *B. zonata* is highly polyphagous and more than 50 plants are affected by this pest such as citrus, fig, apricort, peach, guava and mango and it was first reported in South and Southeast Asia [14]. Another important species of Tephritidae, *B. dorsalis* has been reported to damage more than 100 host plants and is serious pest of several fruits like mango, peach and citrus including

vegetables and fruits from subtropical and tropical regions [16]. It was first originated from Taiwan in 1912 and later its presence was recorded in Asia Pacific region. It cause 80% loses to guava fruits at market level.

Keeping in view the economic importance of fruit flies for citrus, it is important to develop some control strategy that must be economical and ecologically compatible. Among the various tools used for the control of fruit flies, the use of methyl eugenol in traps is considered one of the effective strategies of IPM. Use of methyl eugenol along with the insecticide in traps is called male annihilation technique, because methyl eugenol attracts male fruit flies and insects are ultimately killed, thus reducing the number of males for mating. The most efficient way to capture the fruit flies is use of those traps having combined effect of both olfactory and visual stimuli and these stimuli help in host location [17, 18]. The present study was carried out to determine the preference of fruit flies to different colored traps of methyl eugenol. The traps were installed in citrus orchards and their preferences for fruit flies were determined. Other objectives of this study were to identify the captured species and to determine the population trend of fruit fly in the citrus orchards.

2. Materials and Methods

2.1 Experimental design and layout

Research trial was laid out in the research farm (citrus orchard) of the Citrus Research Institute, Sargodha, Pakistan in 2016 and 2017 (July to September) with eleven treatments (T_1 = Yellow, T_2 = Green, T_3 = Blue, T_4 = Grey, T_5 = Red, T_6 = Black, T_7 = White, T_8 = Orange, T_9 = Yellow green, T_{10} = Transparent and T_{11} = Brown) and three replicates per treatment. Experiment was designed according to RCBD. Eleven traps of different colors were installed in the citrus (Kinnow) orchard in three replicates. Distance of 50 m was maintained between any two traps. Each trap was provided with cotton swab charged with methyl eugenol (0.4 ml) and spinosad (1 ml) placed inside the trap in a loop made of iron wire. Traps were serviced with these chemicals at weekly intervals.

2.2 Collection and identification of fruit flies

The fruit flies were collected from these traps at weekly intervals during the whole trial, counted and identified to the species level.

2.3 Statistical analysis

The data was analyzed by statistix software (version 8.1) using RCBD and significance of means were determined by LSD test at $\alpha = 0.05$. Means were determined on per week basis. The numbers of fruit flies trapped in each treatment were pooled irrespective of species and also species wise. Population trend was determined by counting total number of insects caught in all traps per week during the whole experimental duration. Dominance of different species caught was estimated by measuring their percentages.

3. Results

3.1 Average population of fruit flies caught in different traps on per week basis irrespective of species

Means of fruit flies irrespective of species were determined on per trap/week basis and results are summarized in Table 1. In the year 2016, significantly higher fruit fly catch was observed in yellow (T_1) colored trap (102.29 flies/week), followed by transparent (T_{10}) colored trap (89.26 flies/week) and green (T_2) colored trap (74.98 flies/week). Significantly lower but statistically similar catching efficiency was observed in black (T6, 29.33 flies/week) and brown (T11, 19.55 flies/week) colored traps. In the year 2017 yellow (T_1, 54.90 flies/week) and transparent (T_{10}, 58.09 flies/week) colored traps showed statistically similar but maximum fruit fly catch as compared to other colored traps, however the lowest but statistically similar catching efficiency was recorded for blue (T_3, 25.01 flies/week), grey (T_4, 21.87 flies/week), black (27.88 flies/week), white (T_7, 29.17 flies/week) and brown (T_{11}, 29.17 flies/week) colored traps. Pooled data of both years also showed that catching efficiency of yellow (T1, 78.59 flies/week) and transparent (T10, 73.68 flies/week) traps remained statistically similar when compared with themselves but it was significantly higher as compared to all other treatments. Lowest but statistically similar fruit fly catch was observed in grey (T4, 28.17 flies/week), black (T6, 28.61 flies/week) and brown (T11, 24.36 flies/week) colored traps.

Table 1: Mean number of fruit flies (irrespective of species) caught in different colored traps on per week basis in the citrus orchard for the year 2016, 2017, and means of both years (July-Sep).

Treatments[a]	Number of fruit flies/trap/week ± SE[b]		
	Year 2016	Year 2017	Pooled data of both years
T1 (Yellow)	102.29 ± 28.33 A	54.90 ± 4.55 AB	78.59 ± 16.43 A
T2 (Green)	74.98 ± 12.25 BC	29.98 ± 1.48 CDE	52.48 ± 6.71 B
T3 (Blue)	41.43 ± 5.94 DEF	25.01 ± 1.70 E	33.22 ± 3.66 CDE
T4 (Grey)	34.52 ± 5.51 DEF	21.87 ± 0.89 E	28.17 ± 2.54 DE
T5 (Red)	45.64 ± 2.22 DEF	38.08 ± 2.47 CD	41.86 ± 0.34 BCD
T6 (Black)	29.33 ± 5.17 EF	27.88 ± 5.47 DE	28.61 ± 2.17 DE
T7 (White)	51.24 ± 4.40 CDE	29.17 ± 3.89 DE	40.20 ± 3.50 BCDE
T8 (Orange)	57.48 ± 1.39 CD	24.95 ± 0.53 E	41.21 ± 0.93 BCD
T9 (Yellow green)	50.62 ± 4.72 CDE	42.45 ± 2.16 BC	46.54 ± 2.83 BC
T10 (Transparent)	89.26 ± 16.50 AB	58.09 ± 12.04 A	73.68 ± 14.27 A
T11 Brown	19.55 ± 1.81 F	29.17 ± 6.38 DE	24.36 ± 2.32 E
P-value	0.0001<	0.0001	0.0001<
F-value	8.02	7.86	9.32

[a]Each treatment consist of colored traps of three replicates

[b]Standard Error

Means sharing similar letter (s) in a column are statistically non-significant at $P>0.05$ (LSD test)

3.2 Average population of *B. zonata* caught in traps on per week basis

The average number of fruit flies of *B. zonata* caught in different colored traps on per week basis is given in table 2. The data of year 2016 and 2017 shows that maximum population of *B. zonata* was recorded in yellow (T_1, 91.81 and 50.50 flies/week) and transparent (T_{10}, 78.07 and 53.55 flies/week). Pooled data of both years also show that yellow (T_1, 71.15 flies/week) and transparent (T_{10}, 65.81 flies/week) colored traps attracted statistically maximum population of *B. zonata* as compared to all other colored traps, while the catch of grey (T_4, 25.20 flies/week) black (T_6, 25.91 flies/week) and brown (T_{11}, 22.18 flies/week).

Table 2: Average Number of *Bactrocera zonata* caught in different colored traps on per week basis/per trap in the citrus orchard for the year 2016, 2017, and means of both years (July-Sep).

Treatments[a]	Number of fruit flies/trap/week ± SE[b]		
	Year 2016	Year 2017	Pooled data of both years
T1 (Yellow)	91.81 ± 25.28 A	50.50 ± 4.72 A	71.15 ± 14.94 A
T2 (Green)	66.67 ± 10.40 BC	27.37 ± 1.53 BCD	47.02 ± 5.80 B
T3 (Blue)	36.38 ± 4.20 DEF	23.21 ± 1.79 CD	29.80 ± 2.78 CDE
T4 (Grey)	30.45 ± 4.49 DEF	19.96 ± 0.60 D	25.20 ± 2.14 DE
T5 (Red)	40.29 ± 2.23 DEF	33.86 ± 1.72 BC	37.08 ± 0.39 BCD
T6 (Black)	25.95 ± 4.83 EF	25.88 ± 5.39 CD	25.91 ± 2.29 DE
T7 (White)	45.21 ± 4.20 CDE	26.65 ± 3.66 BCD	35.94 ± 3.21 BCDE
T8 (Orange)	51.74 ± 1.29 CD	21.93 ± 0.37 CD	36.83 ± 0.66 BCD
T9 (Yellow green)	45.14 ± 3.53 CDE	38.20 ± 2.00 B	41.67 ± 2.50 BC
T10 (Transparent)	78.07 ± 12.05 AB	53.55 ± 11.27 A	65.81 ± 11.66 A
T11 Brown	17.86 ± 1.54 F	26.50 ± 5.19 BCD	22.18 ± 1.87 E
P-value	0.0001<	0.0001	0.0001<
F-value	8.27	7.78	10.43

[a]Each treatment consist of colored traps of three replicates
[b]Standard Error
Means sharing similar letter (s) in a column are statistically non-significant at *P>0.05* (LSD test)

3.3. Average population of *B. dorsalis* caught in traps on per week basis

Catching efficiency of different colored traps for *B. dorsalis* is given in table 3. In the year 2016, statistically similar but maximum population of *B. dorsalis* was trapped in yellow (T1, 10.43 flies/week), green (T2, 8.31 flies/week) and transparent (T10, 9.27 flies/week) colored traps, while all other treatments showed significantly lower catching efficiency with least population in the grey (T4, 4.00 flies/week), black (T6, 3.26 flies/week) and brown (T11, 1.69 flies/week) colored traps. In the year 2017, yellow and transparent traps did not differ statistically in catching efficiency of B. dorsalis showed maximum population as compared to other traps. Mean data of both years for *B. dorsalis* catch also shows that yellow (T_1, 7.30 flies/week) and transparent (T_{10}, 7.08 flies/week) traps produced statistically significant results by catching the maximum population as compared to all other traps. Least population catch of *B. dorsalis* was observed for blue (T3, 3.39 flies/week), grey (T4, 2.88 flies/week), black (T6, 2.90 flies/week) and brown (T11, 2.28 flies/week) colored traps.

Table 3: Average Number of *Bactrocera dorsalis* caught in different colored traps on per week basis/per trap in the citrus orchard for the year 2016, 2017, and means of both years (July-Sep).

Treatments[a]	Number of fruit flies/trap/week ± SE[b]		
	Year 2016	Year 2017	Pooled data of both years
T1 (Yellow)	10.43 ± 3.18 A	4.15 ± 0.39 AB	7.30 ± 1.56 A
T2 (Green)	8.31 ± 1.90 ABC	2.42 ± 0.16 CD	5.37 ± 1.02 B
T3 (Blue)	5.04 ± 1.77 CDE	1.74 ± 0.11 D	3.39 ± 0.90 CD
T4 (Grey)	4.00 ± 1.09 DE	1.76 ± 0.21 D	2.88 ± 0.51 CD
T5 (Red)	5.43 ± 1.05 CD	3.32 ± 0.27 BC	4.38 ± 0.40 BC
T6 (Black)	3.26 ± 0.63 DE	2.54 ± 0.58 CD	2.90 ± 0.20 CD
T7 (White)	6.02 ± 0.32 BCD	2.33 ± 0.39 CD	4.18 ± 0.34 BC
T8 (Orange)	5.50 ± 1.18 CD	2.24 ± 0.55 CD	3.87 ± 0.82 BCD
T9 (Yellow green)	5.48 ± 1.30 CD	3.32 ± 0.19 BC	4.40 ± 0.59 BC
T10 (Transparent)	9.27 ± 1.49 AB	4.88 ± 0.75 A	7.08 ± 1.11 A
T11 Brown	1.69 ± 0.27 E	2.87 ± 1.08 BCD	2.28 ± 0.41 D
P-value	0.0011	0.0015	0.0005<
F-value	4.99	4.77	8.43

[a]Each treatment consist of colored traps of three replicates
[b]Standard Error
Means sharing similar letter (s) in a column are statistically non-significant at *P>0.05* (LSD test)

3.4 Trend of fruit fly population

In the year 2016, fruit fly population in the citrus orchards showed an increasing trend from start of July to August, with peak population in the last week of August. After that, population started to decline and approximately remained constant in the whole month of September (Figure 1). However, in the year 2017, two peaks were observed; 1st in the 3rd week of the August and 2nd in the 3rd week of September (Figure 2).

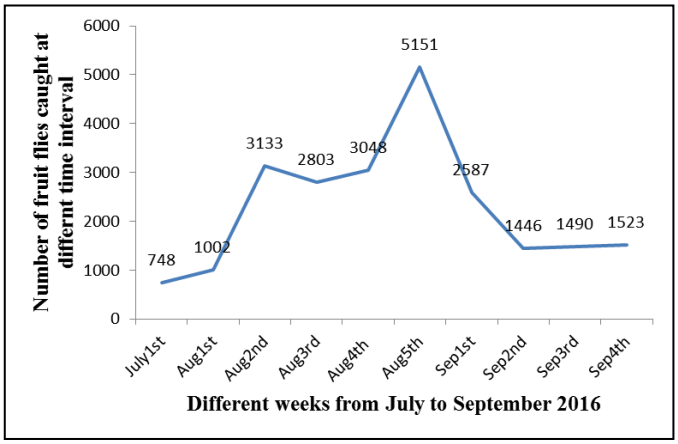

Fig 1: Fruit fly population trend in different weeks from July to September 2016.

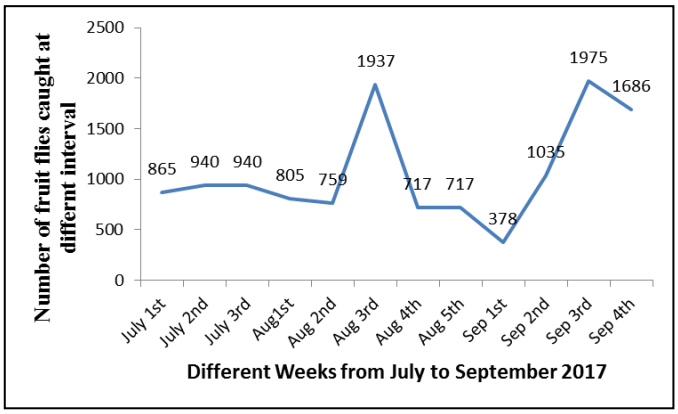

Fig 2: Fruit fly population trend in different weeks from July to September 2017.

3.5. Dominance of fruit fly species

It was found that percentage of *B. zonata* was 89.14% and 91.66 while that of *B. dorsalis* was 10.85% and 8.33%, in the years 2016 and 2017, respectively (Figure 3).

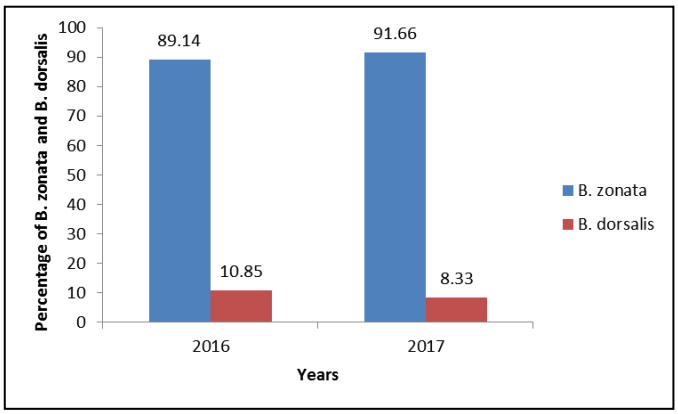

Fig 3: Percentage of different fruit fly species in citrus orchards.

4. Discussion

The family Tephritidae contains fruit flies which act as serious threat to production of various fruits and vegetables such as citrus, mango, guava and cucurbits and the species which are of international significance are *B. zonata*, *B. dorsalis* and *B. cucurbitae* [19]. The present study was done to test the color preferences of fruit flies in citrus by installing different colored traps of methyl eugenol. Our findings revealed that fruit flies (irrespective of species and also species wise) in citrus orchards of Sargodha, Pakistan showed maximum preference for yellow and transparent traps colored traps, while the least preferred colors were grey, black and brown. Previously, Ravikumar and Viraktamath [20] also reported that yellow (70.45 fruit flies/trap/week) and transparent (5.13 fruit flies/trap/week) colored traps were most preferred by *B. correcta* in guava and mango orchards, respectively. Jalaluddin *et al.* [21] and Madhura [22] have also reported the attraction of *Bactrocera* spp. to yellow colour traps. Prokopy and Owens [17] have also found yellow color the most attractive one to Tephritid fruit flies. The most probable reason for the preference of fruit flies species to yellow colour traps might be due to reflection of yellow colour [23]. Robacker *et al.* [24] also studied that green and yellow were the most attractive colors to Mexican fruit flies, *Anastrepha ludens* while black, red, blue, and white traps were not more attractive than colorless control traps.

In this work, to determine the temporal distribution of fruit flies in citrus orchards, the population trend of fruit flies was also studied. In the year 2016, a gradual increase in the population was observed from July to August and population started to decline from start of September. However, in the year 2017, we have observed two peaks of fruit fly populations; one in the August and other in the month of September. Similar to our study, Mahmood and Mishkatullah [25] also reported peak population of fruit flies during the month of August in guava orchards. Gillani *et al.* [26] also reported the increase in population trend of fruit flies from May to August with peak population in the month of July in the guava orchards of Pakistan. Nandre and Shukla [27] also studied the population dynamics of fruit fly, *B. dorsalis* on sapodilla *Manilkara achras* and observed that the fruit fly population prevailed throughout the year with maximum activity (172.1 flies per trap) during March to August. This study also reports that *B. zonata* was found in the citrus orchards with maximum percentage of 89.14%-91.66% while the dominance of *B. dorsalis* in the citrus orchards of Sargodha was only 8-10%.

5. Conclusion and Recommendations

It can be concluded from this study that timely monitoring is important for effective control of fruit flies using traps mainly yellow colored trap or combination of yellow traps with transparent traps. The traps must remain installed in the peak season of fruit fly populations in the citrus orchards to avoid heavy losses (e.g. July to September). Timely installation of these traps in citrus orchards will help farmers in detection and control of fruit flies at preliminary stages and thus result in significantly much reduced population density at peak season of citrus crops. This strategy should be implemented as part of integrated pest management by citrus growers to minimize the huge damage caused by fruit flies. Other management practices such as orchard sanitation, weed control, application of bait sprays, hoeing under the canopy and picking and burying of dropped fruits should also be incorporated as a part of IPM in the citrus orchards to achieve the satisfactory results for the control of fruit flies.

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