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Bait attractants based on artificial fruit-essence for trapping and monitoring *Drosophila suzukii* (Diptera: Drosophilidae) females in Peshawar-Pakistan

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Abstract The spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), native to Asia was reported for the first time in Pakistan in 2005 from Islamabad. Following the recent detection of *D. suzukii* in cherry orchards at Kalam (Khyber Pakhtunkhwa) a trapping system was developed to monitor it on fruits. The efficacy of three baited trap attractants using apple cider vinegar, yeast sugar solution and synthetic fruits-essence solution (using banana, guava or strawberry as fruit) were tested. Traps were installed at fruit markets at three different locations in Peshawar. The bait based on synthetic fruit-essence solution yielded the highest captures. Among the baits based on synthetic fruit-essence solution, the highest captures were obtained with strawberry essence.

Keywords Bait traps · Fruit essence · Attractant · Strawberry · Flies capture

Introduction

The spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is a serious pest of fruit crops. Unlike most other *Drosophila* species, it is able to oviposit and feed on healthy fruits (Davis et al. 2010; Cini et al. 2012). Native to South-East Asia, the species was first detected in Japan in 1916 (Kanzawa 1939; Cini et al. 2012). Since then, *D. suzukii* has rapidly spread to other countries in Asia (Hauser et al. 2009; Amin ud Din et al. 2005; Cini et al. 2012) as well as Europe, Central and North America (Hauser et al. 2009). Worldwide, *D. suzukii* is now considered as an important pest causing considerable economic damage to agriculture (Mitsui et al. 2010; Walsh et al. 2011; Cini et al. 2012).

Drosophila suzukii is currently monitored using traps baited with a variety of attractants available commercially in different regions of the world. Two commonly used baits are based on apple-cider vinegar and yeast-sugar solution (Knight et al. 2013; Iglesias et al. 2014). However, these baits are not selective and sufficiently efficient for monitoring *D. suzukii* populations, especially females (Becher et al. 2012; Lee et al. 2012; Kleiber et al. 2014; Burrack et al. 2015). There is therefore a need to develop a more efficient and a more specific bait to attract *D. suzukii*, exploiting the most active volatiles among emitted by those fruits that serve as host of *D. suzukii*.

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Most of drosophilids searching for host fruits use their volatile compounds as olfactory cues (Arx et al. 2011) and host fruits aroma play a major role in attracting them for oviposition (Linz et al. 2013) and other activities such as feeding and mating (Faucher et al. 2013). In general, *Drosophila* spp. are highly attracted towards yeast and volatile cues emitted by fermenting host fruits (Becher et al. 2012; Landolt et al. 2012; Steck et al. 2012). *Drosophila suzukii* females have shown attraction towards the odors emitted by fresh berry and cherry fruits (Revadi et al. 2012), among which, strawberries and raspberries odors induced a good attraction linked with a positive antennal response (Abraham et al. 2015). Strawberry fruits, when damaged, can release odors that serve as stimuli for *D. suzukii* attraction and oviposition (Bernardi et al. 2017). Bhatia (2016, unpublished) from India has recently shown that the use of artificial-fruit essences can play crucial role in such pest trapping. In this context, the present study aims to identify a synthetic fruits-essence as an attractant that can be routinely used to trap and monitor *D. suzukii* in the farmers' community in the temperate regions of Pakistan.

Materials and methods

Insects

A colony of *D. suzukii* maintained in the laboratory of the Department of Plant Protection of the University of Agriculture of Peshawar was used for laboratory tests. The flies initially collected from rotten fruits were reared continuously in cages of 0.4 m × 0.4 m × 0.6 m. The larvae fed on artificial diet of Dalton et al. (2011) and the adults on a solution of sucrose and water. Wet cotton wools were placed inside the rearing cage to maintain humidity. The rearing conditions were at (mean ± SE) 22 ± 0.25 °C and 94 ± 0.34% relative humidity with 10 h:14 h (L: D) photoperiod.

Bait attractants

The baits containing three different artificial fruit-essences were prepared by mixing 25 g of sugar (Hunza Sugar Mills Limited, Faisalabad) with 12 g of dry yeast (Al-Tabakh Instant Dry Yeast, Alham Bighzada Manufacturing Company Limited, Iran) in 500 mL of distilled water. To this solution, 10 mL of synthetic fruits

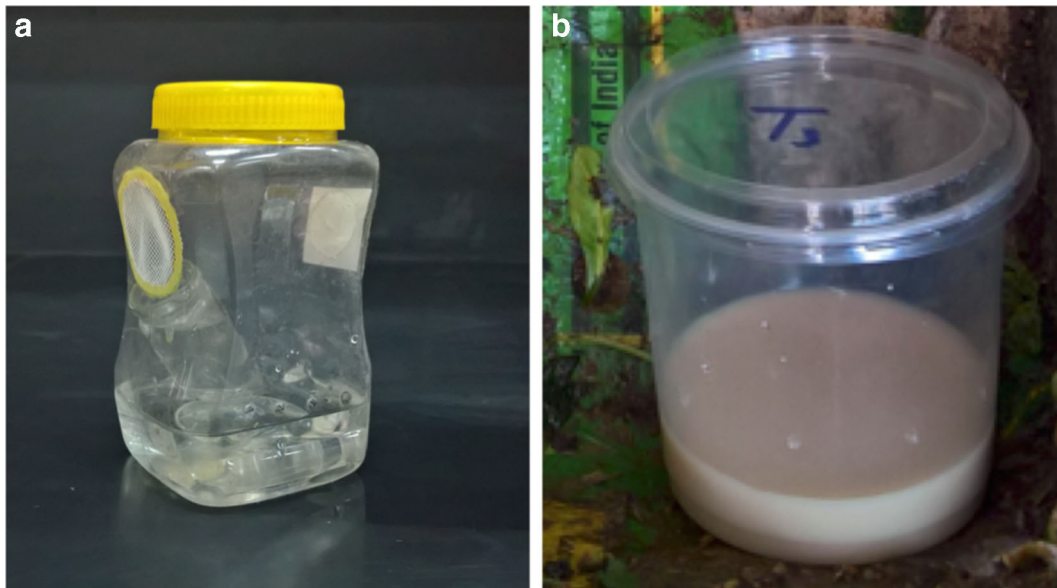
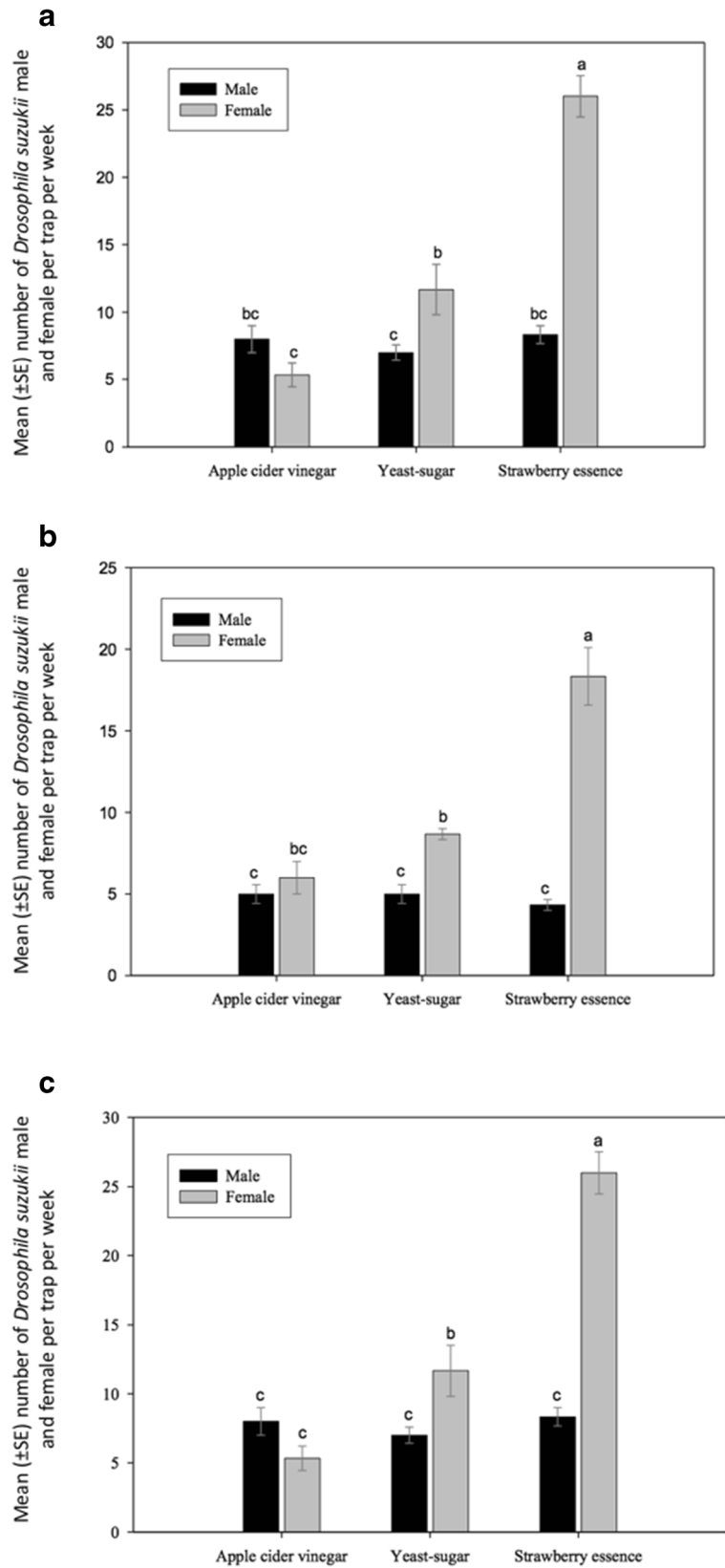


Fig. 1 Photos of traps designs used in (a) laboratory experiment for treatments comparison having a small vial containing treatment bait suspended in detergent water; in (b) field tests with small

diameter holes just above the treatment bait for direct entry of spotted wing drosophila

Fig. 2 Mean (\pm SE, $n = 3$) numbers of *Drosophila suzukii* males (black bars) and females (grey bars) trapped in each bait in (a) Board bazar, (b) Firdos bazar and (c) Nasirpur market of Peshawar. Bars with different letters are significantly different at 5% level according to Fishers' LSD test following ANOVA



essence of either strawberry, guava or banana (Standard Manufacturing Company, Lahore) were added.

Another bait of yeast-sugar solution was prepared by mixing 39.4 g of sugar (Hunza Sugar Mills Limited, Faisalabad) with 14.8 g of dry yeast (Al-Tabakh Instant Dry Yeast, Alham Bighzada Manufacturing Company Limited, Iran) in 710 ml of water (Iglesias et al. 2014).

Finally, the bait of apple-cider vinegar consisted of a mere solution of 5% acetic acid (Natural Apple cider Vinegar, American Garden Company, New York, USA).

Laboratory tests

For laboratory tests, only the baits containing the three aforementioned different artificial fruit-essences (strawberry, guava or banana) were used to identify the most efficient fruit-essence bait to be used in the field. About 20 mL of fruit-essence attractant was deposited in small 30 mL vials which were placed individually in clear plastic bottle of 200 mL (punched with small holes to allow the entrance of flies) filled at the bottom by 50 mL of odorless soapy water (a mixture of liquid dish soap [Lemon Max, Colgate-Palmolive, Pakistan] with water), constituting the trapping device as shown in Fig. 1a (Birmingham et al. 2011). The control bait trap consisted of 50 mL of soapy water. The tests were performed in the laboratory using 0.6 m × 0.6 m × 0.6 m mesh cages at room temperature between 18 and 24 °C with 10 h:14 h (L: D) photoperiod, which contained wet cotton wools to maintain humidity around 65%. Each test consisted of 50 *D. suzukii* adults (25 females and 25 males) in a single cage containing a solution of sucrose and water. In each cage, the four bait traps to be tested (i.e. 3 containing synthetic fruits essence of strawberry, guava or banana and the control) were placed randomly in each corner of the cage. Every 24 h each trap was checked and rotated. The total number of flies captured in each bait trap was recorded for three consecutive days. The tests were replicated four times.

Field tests

The field tests were conducted in three market places of Peshawar: Board Bazar: N 34°0'0", E 71°28'12"; Firdos Bazar: N 34°0'36", E 71°34'12"; Fruit Market Nasirpur: N 34°01'12", E 71°41'24". In these tests, the most efficient artificial fruit-essence identified in the laboratory tests (see results, i.e. strawberry-essence bait) was used and compared to the known standard baits of apple-cider

Table 1 Percentage (mean^a ± SE, n = 4) of *Drosophila suzukii* adults captured in each bait trap under laboratory conditions

Baits tested	% flies captured
Banana essence	23.71 ± 0.72b
Guava essence	4.99 ± 0.33c
Strawberry essence	71.31 ± 0.60a
Control	0.00 ± 0.00d

^a Means followed by different letters are significantly different at 5% level according to Fishers' LSD test

vinegar and yeast-sugar solution of Knight et al. (2013) and Iglesias et al. (2014), respectively. Another trapping device (Fig. 1b), frequently used in the field, with either artificial fruit-essence, apple-cider vinegar and yeast-sugar solution baits were placed randomly at each location. All *D. suzukii* adults captured in each trap were properly identified (Markow and O'Grady 2006; Vlach 2010) and counted on weekly intervals and the solution was also replaced weekly. Males and females in each treatment sample were separated using the keys of Markow and O'Grady (2006) and Vlach (2010) and counted. Each field test in each location lasted 3 weeks and was repeated three times.

Statistical analysis

For laboratory tests, the percentage of flies trapped in each trap (i.e. number of flies captured in each trap/total number captured × 100) was calculated. Percentages were arcsin transformed to normalize the data and then

Table 2 Total number of *Drosophila suzukii* (mean^a ± SE, n = 3) captured weekly in each bait trap in three different market places of Peshawar

Bait tested	Locations		
	Board bazar	Firdos bazar	Nasirpur market
Apple cider vinegar	13.3 ± 1.8c	11.0 ± 0.6b	18.3 ± 1.2b
Yeast-sugar	18.7 ± 2.4b	13.3 ± 0.9b	22.3 ± 2.2b
Strawberry-essence	33.3 ± 0.9a	22.7 ± 2.0a	37.0 ± 0.6a
Results of 2-ways ANOVA:		Factors	P values
		Bait	<0.001
		Location	<0.001
		Bait x Location	0.0826

^a Within each column, means followed by different letters are significantly different at 5% level according to Fishers' LSD test

subjected to analysis of variance (ANOVA). Means were separated using a Fishers' Least Significant Difference test following the ANOVA and untransformed means are presented in tables.

For field tests, the number of adult flies (both males and females) captured in each trap were used. The data were first normalized by $\ln[x]$. The means were separated by comparing after 2-ways ANOVA by Fishers' LSD test and the untransformed means are presented in the Fig. 2.

All statistics were done using SPSS 20.0 package.

Results and discussion

Under laboratory conditions, strawberry-essence bait attracted significantly more *D. suzukii* adults than the other baits tested (Table 1; results of ANOVA: $F_{3,12} = 4285$, $P < 0.001$) verifying results by Goodhue et al. (2011) and Arno et al. (2012) who found strawberry fruits to be good host. Similarly, Abraham et al. (2015) found strawberries to be more attractive to *D. suzukii* than blueberries and cherries. Still, *D. suzukii* was shown to be attracted towards fruit volatiles of raspberry, strawberry, blueberry and cherry, indicating the importance of fruit odours in providing cues for host selection (Revadi et al. 2015a, b).

In the field tests, fly captures differed between baits and locations (Table 2). Regardless of the location, the highest number of *D. suzukii* adults was captured in the strawberry-essence bait traps, followed by yeast-sugar solution, while apple cider vinegar attracted the least numbers. Significantly more *D. suzukii* adults were captured in traps located at Nasirpur Market followed by Firdos bazar and traps at Board bazar received the least numbers of adult flies (Table 2).

In addition, regardless of the location a significant higher number of females than males were captured in strawberry-essence bait traps while with other baits this difference in sex captured was less pronounced (Fig. 2).

In both laboratory and field tests, strawberry-essence solution was found to be the most attractive bait whereby females were more sensitive to this odour than males. This confirms results by Lee et al. (2011), Goodhue et al. (2011), and Arno et al. (2012). Likewise, Revadi et al. (2015a, b) showed that *D. suzukii* was generally attracted to fruit odours. Most insects searching for fruits use their volatile compounds as olfactory cues (Arx et al. 2011). Thus, host fruits aroma plays a major role in orienting drosophilids for oviposition (Linz et al. 2013; Keesey et al. 2015).

In conclusion, strawberry-essence bait trap has a promising effect in attracting *D. suzukii* especially the female flies and thus constitutes a good candidate for monitoring and further research on *D. suzukii* control.

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Compliance with ethical standards

Conflict of interest statement The authors declare that they have no conflict of interest.

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