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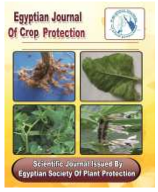

Oviposition preference of spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) for fresh fruit and fruit-like shapes artificial diets of raspberry and blueberry

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ABSTRACT

The spotted wing drosophila (SWD), *Drosophila suzukii* (Matsumura), is a serious insect that attacks a wide range of small skinned. It possesses a serrated ovipositor that allows it to lay eggs in unwounded commercial fruits, causing severe crop losses. This study was implemented to assess the oviposition preference, development time of immature and mature stages and egg hatchability percentages for *D. suzukii* on raspberry and blueberry fresh fruits and fruit-like shapes made of artificial diets in no-choice and choice tests. The results demonstrated that *D. suzukii* females exhibited a preference for laying eggs in fresh fruits over fruit-like shapes, regardless of whether it was raspberry or blueberry in the choice test. However, no significant differences were observed in the number of eggs laid between fresh raspberries and fruit-like raspberry shapes in the no-choice test. Additionally, fresh raspberry and blueberry fruits displayed a significantly two-fold larger diameter compared to their respective fruit-like shapes. Moreover, the choice test results for either raspberry or blueberry indicated that the highest numbers of *D. suzukii* larvae, pupae, and total emerged adults were found on fresh raspberry fruits, while the lowest numbers were recorded on fruit-like blueberry shapes. However, these numbers did not significantly differ from those of fresh blueberry fruits or fruit-like raspberry shapes. Furthermore, the development time for larvae, pupae, and adults was consistently longer when using fruit-like blueberry shapes compared to other treatments, while it was shortest when fresh raspberry fruits were employed.

Key words: *Drosophila suzukii*, Oviposition Preference, Fruit-like Shape, Raspberry, Blueberry, Artificial Diet.

INTRODUCTION

The fruit fly spotted wing drosophila *Drosophila suzukii* (Matsumura) is an invasive economic polyphagous pest infests a wide range of fruit crops including cherries, raspberries, blackberries, blueberries, strawberries, peaches, plums, and grapes (Mitsui *et al.,* 2010; Lee *et al*., 2011a; Cini *et al.,* 2012; Bellamy *et al.,* 2013; Cini *et al.,* 2014; Asplen *et al.,* 2015; Hamby & Becher, 2016). It's native to Asia, introduced pets in Europe and North and South America (Lee *et al.,* 2011a, 2012; Asplen *et al.,* 2015). *Drosophila*

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suzukii possess two unique characteristics capable her to be an onerous pest; its propensity for favoring ripening (as opposed to overripe) fruit (Mitsui *et al.* 2006) and the prominent serrated ovipositor of the female, which upon insertion can cause physical damage to the host fruit.

Very often oviposition wounds, including those caused by Drosophila flies, provide access to secondary infection by both insects and pathogens including fungi, yeasts, and bacteria causing additional losses (De Camargo and Phaff 1957, Molina *et al.* 1974, Louise *et al.* 1996). Eggs develop into larvae within the fruit, causing them to become soft and rot rapidly, resulting in reduced crop yields and significant financial losses. Insect preference of host plants is affected by various factors, such as chemical and visual cues, enemy avoidance, plant chemistry, morphology, or nutritional requirements of insect life stages (Lee, 2007; Webster *et al*., 2012).

A previous study demonstrated that female *D. suzukii* are attracted to odors from fresh raspberry, blackberry, blueberry, strawberry, and cherry in two-choice assays (Revadi *et al.,* 2015a), and these fruits may become susceptible to attack once fruit coloration begins (Lee *et al.,* 2011a), with this pest strongly preferring the blushing and ripe fruit stages (Karageorgi *et al.,* 2017).

Phytophagous insects mostly choose suitable host plants for oviposition and feeding to optimize offspring success (Bernays and Chapman, 1994; Cunningham, 2011). Other studies have shown that generalist insect females sometimes oviposit on sites that are not always optimal for larval survival (Cunningham, 2011). Oviposition site selection is a critical factor in determining the survival rate of offspring in insect species. A nutritionally suitable resource may be heavily used by other insects and the offspring may suffer from intense competition (Sato *et al*., 2021). Because many other closely related Drosophila species lay eggs onto fermented fruits, this behavior allows *D. suzukii* to use a carbohydrate-rich resource before interspecific competition becomes intense (Silva-Soares *et al*., 2017 and Young *et al.,* 2018). The behavioral change to deposit eggs into ripening fruits must have been related to changes in the sensory systems plus the changes in the ovipositor morphology to evaluate the oviposition substrate (Sato *et al*., 2021).

Drosophila suzukii greatly preferred ripe fruit to rotten fruit, however *Drosophila melanogaster* showed an opposite propensity and preferred rotten fruit (Karageorgi *et al.,* 2017, Lee *et al*., 2011a and *Kienzle et al.,* 2020). Consequently, it has expanded the potential substrates range to include different degrees of hardness and does not essentially prefer a harder fruit surface (Burrack *et al.,* 2013 and Lee *et al.,* 2016). Oviposition selection site depending on the fruit condition involving an integration of multiple sensory cues. *Drosophila suzukii* depends on olfactory cues associated with oviposition sites like other drosophilids (Hamby and Becher, 2016; Kirkpatrick *et al.,* 2016; Takahara and Takahashi, 2017). These odor cues are mostly correlated with a distinguish resource such as fungi or fallen or ripening fruits for a specific Drosophila species, with following evolutionary adaptations to the olfactory system that further enforce and boost movement to the chemical distinct resources (Keesey *et al.,* 2015).

Additionally, more information about fruit and substrate textures probably needs to be understood to make final decisions by pests. *Drosophila melanogaster*, using mechanosensory (texture) and chemosensory (taste) information for feeding and oviposition decisions (Jeong *et al.,* 2016 and Zhang *et al.,* 2020). Determining preferential responses of *D. suzukii* females could be helpful for infestations severity predication (Kalaitzaki *et al.,* 2013; Macfadyen and Muller, 2013) and provide opportunities for behavioral pest manipulation based on landscape management (Bianchi *et al.,* 2008; Srinivasan *et al.,* 2013).

This study aims to investigate the *D. suzukii* oviposition site preferences and selection between fresh fruit and fruit-like shape, also the immature stages development and survival on both.

MATERIALS AND METHODS

1. *Drosophila suzukii* rearing colony

A colony of *D. suzukii* was maintained in a growth chamber at 24 ± 1^{o}C, 50-60% RH, and 16L:8D photoperiod. This was initiated by collecting emerged flies from wild fruit collected in Van Buren and Allegan counties, Michigan, USA. The insects were reared in plastic vials (25 mm diameter, 95 mm high) with a foam vial plug filled with 2 cm of standard cornmeal artificial diet as a substrate for oviposition. Flies were transferred to fresh vials 2-3 times weekly to provide new egg-laying substrate. Flies used in all experiments were 7–10 days old.

2. No-choice and choice tests

Organic raspberries and blueberries were brought from a local grocery store and washed with tap water, then left on paper towels to get dry. We used raspberry and blueberry-based agar substrates for oviposition assays. A raspberry and blueberry purees substrates were made by blending whole, ripe berries separately (Driscolls organic, USA origin) with equal parts water, and 1% each of agar, and the anti-fungal agents: Tegosept (methyl paraben [CAS: 99-76-3]), and propionic acid (CAS:79-09-4). The mixture is left to cool, then added to a squeeze bottle and poured on raspberry and blueberry molds silicone to form fruit-like shape (Figure 1).

In no-choice test, five raspberry fruit-like shape artificial diets were placed in a Petri-dish (60 mm) inside a 32oz small plastic container, has a small circle hole closed with a foam plug to ease flies introducing and sealed with a lid have a small area covered with mesh. In another small plastic container, five fresh raspberry fruits were placed in a Petri-dish (60 mm). Each treatment was replicated 5-6 times. Ten males and 10 females of *D. suzukii* aged 7-10 days were released inside each container for 24 hrs for oviposition. The same steps were carried out for blueberries (2 treatments: the first treatment is blueberry fruit-like shape artificial diets, and the second treatment is fresh blueberry fruits) with 5-6 replicates for each treatment.

The number of eggs laid were counted and observed to figure out the egg hatchability for each replicate. All containers were kept in a growth chamber (24 ± 1^{o}C, 50-60% RH and 16L:8D photoperiod). The neonate larvae were counted and monitored until pupation.

The number of pupae were also counted and recorded and moved to a Petri-dish provisioned with a moisture paper towel on its base and overseen till adult emergence. The adult flies were counted and sexed. The larval, pupal, and adult development times were determined.

Raspberry and blueberry were separately assayed in a choice test (Figure 1). Five fruit-like shape artificial diets and five fresh fruits were placed in a 710 ml plastic container designed with the same way of the small plastic container which used in no-choice test. Ten males and 10 females of *D. suzukii* were introduced to each container (5-6 replicates) for 24 hrs. The number of eggs laid were counted in fresh fruits and fruits-like shape and recorded. The egg hatchability percentage was calculated. All containers were kept in a growth chamber (24 ± 1^{o}C, 50-60% RH and 16L:8D photoperiod) and checked daily to record other parameters as mentioned above in no-choice test (Figure 2).

3. Fruit diameter measurement

The diameter for all raspberry and blueberry fresh fruits and fruit-like shape/ replicate/ treatment was measured using digimatic caliper in no-choice and choice tests before inserting fruit inside containers.

4. Data analysis

Statistical analyses were conducted using JMP Pro 16 (SAS 2013, Cary, NC). The data for the eggs laid in no-choice and choice tests were analyzed with one-way ANOVA and means were compared using Tukey-Kramer HSD. *D. suzukii* immature development were analyzed with two-way ANOVA followed by Tukey's honestly significant difference (HSD) tests to separate means. Count data were log trans-formed, and percentage data were arcsine transformed to improve normality and homoscedasticity.

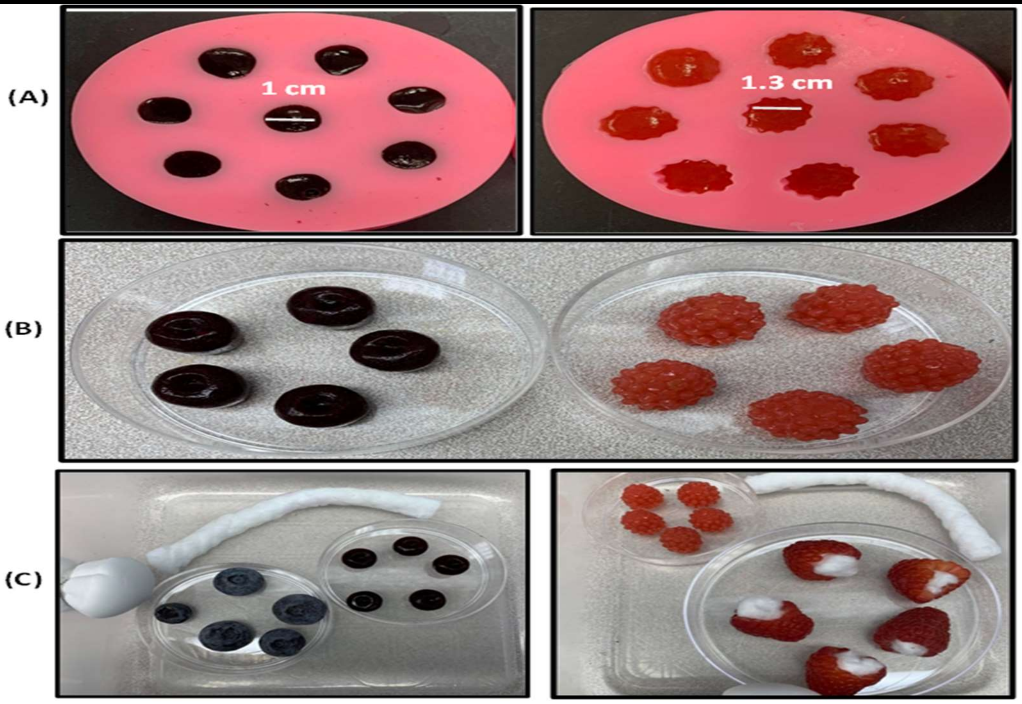

Figure (1): Raspberry and blueberry artificial diet fruit-like shapes: (A) Silicon mold to form blueberry artificial diet fruit-like shapes on the left side and raspberry artificial diet fruit-like shapes on the right side. (B): Blueberry artificial diet fruit-like shapes on the left side and raspberry artificial diet fruit-like shapes on the right side. (C): A plastic container includes a choice test between blueberry fresh fruit and Blueberry artificial diet fruit-like shapes on the left side and raspberry fresh fruit and raspberry artificial diet fruit-like shapes on the right side. Containers were provisioned with moisture wick to increase the humidity inside the container and boost egg oviposition as shown in photo C.

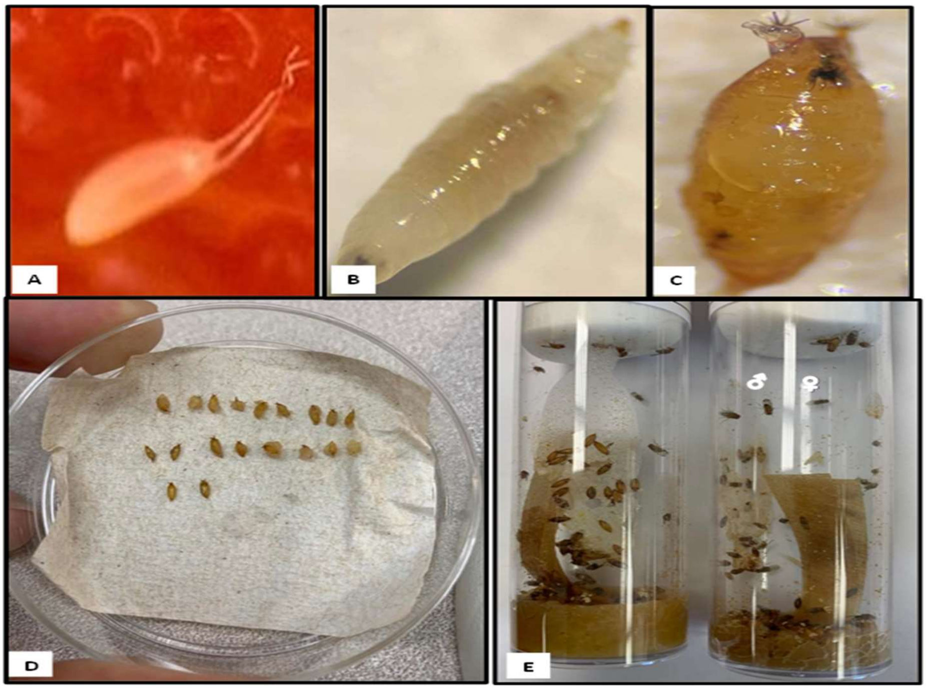

Figure (2): *Drosophila suzukii* life stages (A): egg with two thin respiratory filaments laid in raspberry artificial diet, (B): Third instar larva, (C): Pupa stage, (D): Collected pupae on a paper towel moisture with water and placed in 60 mm Petri dish and (E): *Drosophila suzukii* culture rearing vials with a foam plug and containing a standard cornmeal artificial diet for female oviposition and all stages survival and development until adult emergence (male ♂on the left side has a two black spots on wing, but female ♀ did not has any spots on wings).

RESULTS

1. *Drosophila suzukii* oviposition preference

The number of eggs laid by *D. suzukii* did not significantly differ between treatments for both raspberry (ANOVA-one way, DF=1, F=0.4, P=0.520) and blueberry hosts (ANOVA-one way, DF=1, F=0.05, P=0.836) on no-choice test (Figure 3A). Where the number of eggs laid in raspberry fresh fruit was 94.17 eggs while 105.5 eggs on raspberry fruit-like shape. On blueberry, 92.8 eggs were found on blueberry fresh fruit, however 95.3 eggs were observed on blueberry fruit-like shape. In contrast, choice test showed a great significant difference between treatments for raspberry (ANOVA-one way, DF=1, F=6.6, P=0.034) and blueberry (ANOVA-one way, DF=1, F=11.1, P=0.01) in the number of eggs laid (Figure 3B). Fresh fruits of raspberry significantly showed the greatest number of eggs (107.4 eggs) than raspberry fruit-like shape (42.4 eggs). Similar results were recorded for blueberries, where the highest number of eggs laid on fresh fruit (69.6 eggs) than blueberry fruit-like shape (30.2 eggs).

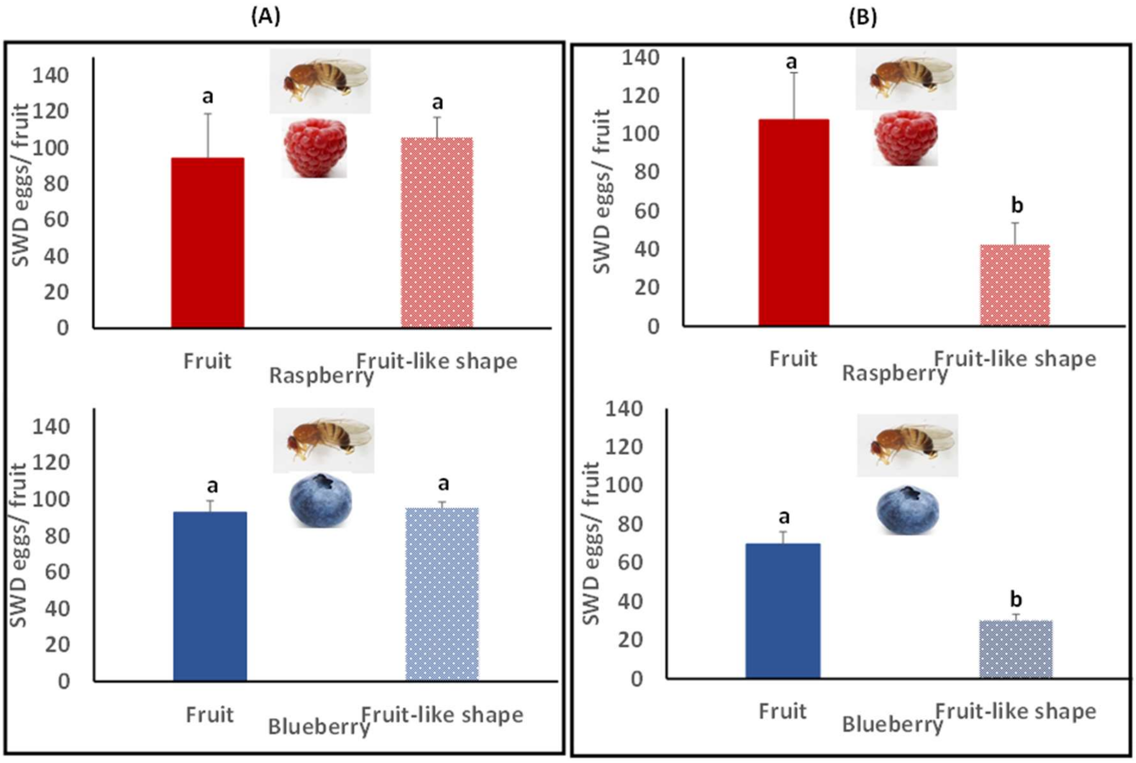

Figure (3): Oviposition preference (no. of eggs laid per 10 females in 24 hr) of *D. suzukii* to fresh fruit vs. fruit-like shape of raspberry and blueberry (A) in no-choice test and (B) in choice test. Bars refer to mean+ SE, and different letters over the bars indicate significant differences (Tukey-Kramer HSD, P <0.05).

The egg hatchability percentage is determined in both no-choice and choice tests for raspberry and blueberry treatments (Figure 4). Results revealed that egg hatchability (%) showed a significant difference between raspberry treatments in no-choice test (ANOVA-one way, DF=1, F=53.2, P< 0.0001). Raspberry fresh fruit showed significantly higher egg hatchability (97.8%) compared to fruit-like shape (80.2%). On the other hand, egg hatchability (%) did not significantly differ between blueberry treatment in no-choice test (ANOVA-one way, DF=1, F=1.0, P=0.326). Where egg hatchability was 53.2% and 56.6% on blueberry fresh fruit and fruit-like shape, respectively.

In the choice test, significant differences were observed between raspberry treatments (ANOVA-one way, DF=1, F=20.2, P=0.0009). Egg hatchability was higher on raspberry fresh fruit (92.5%) than fruit-like shape (74.4%). Similar results were detected for blueberry, where there was a significant difference between treatments in egg hatchability % (ANOVA-one way, DF=1, F=6.6, P=0.026). The highest egg hatchability was noted on blueberry fresh fruit (59.5%) compared to fruit-like shape (46.1%).

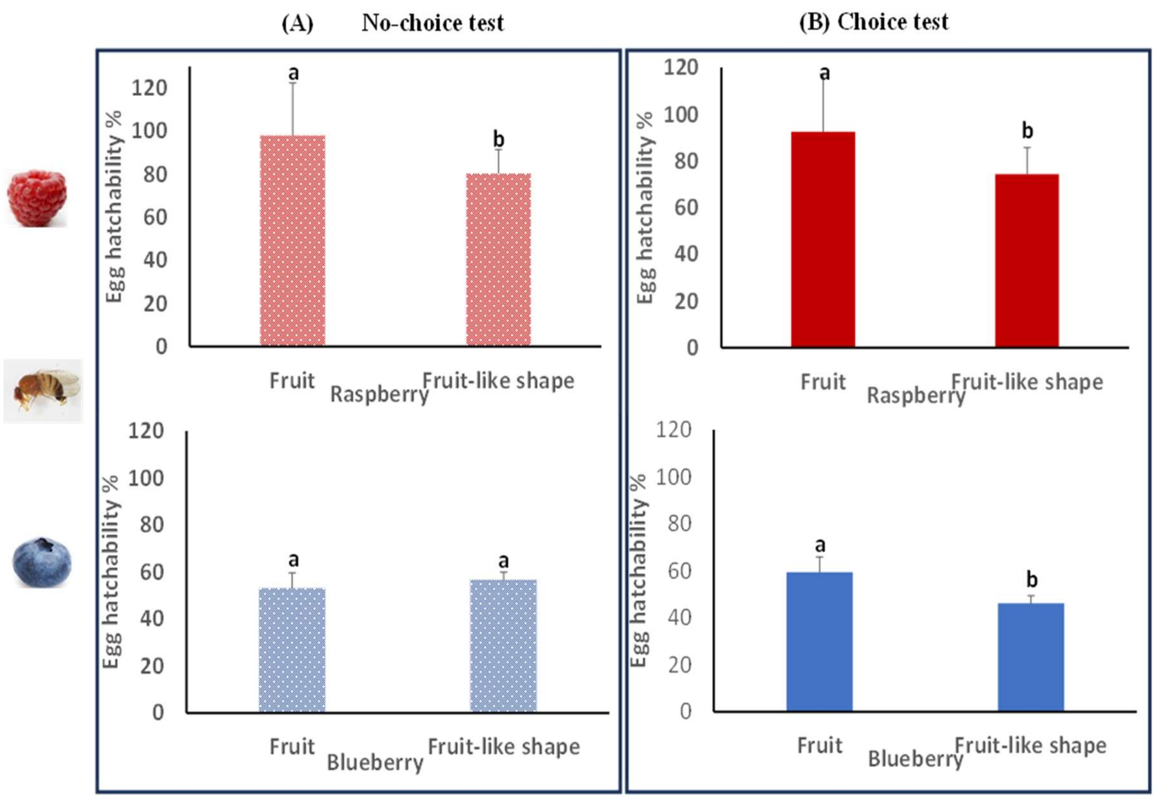

Figure (4): Egg hatchability percent for eggs laid on fresh fruit vs. fruit-like shape for raspberry and blueberry (A) in no-choice test and (B) choice test. Bars refer to mean+ SE, and different letters over the bars indicate significant differences (Tukey-Kramer HSD, P <0.05).

2. Fresh and fruit-like shape fruits diameter

The diameter (mm) of each fresh fruit and fruit-like shaped fruits for raspberry and blueberry was measured and illustrated in (Figure 5). There was a significant difference between treatments for both raspberry (ANOVA-one way, DF=1, F=57.2, P< 0.0001) and blueberry (ANOVA-one way, DF=1, F=141.8, P< 0.0001) in no-choice test. The mean number of raspberry fresh fruit diameter was significantly larger than raspberry fruit-like shape diameter (20.5 mm and 10.1 mm, respectively). Similarly, the diameter of fresh blueberry fruit was greatly larger than blueberry fruit-like shape (17.3 mm and 7.8 mm, respectively). Likewise, there was a significant difference between raspberry treatments (ANOVA-one way, DF=1, F=166.0, P< 0.0001) and blubbery (ANOVA-one way, DF=1, F=420.7, P< 0.0001) in choice test. Significant larger diameter was observed on raspberry for fresh fruit (22.03 mm) than fruit-like shape (9.8 mm). In addition, blueberry fresh fruit had significantly larger fruit diameter (18.1 mm) than fruit-like shape of blueberry (7.2 mm). A correlation between diameter of the fresh fruit and fruit-like shape of raspberry and the number of *D. suzukii eggs* laid (Figure 6).

High correlation was observed between diameter of fruit and number of eggs laid in fresh fruit and fruit-like shape of raspberries on no-choice test (R^2=0.620 and 0.468, respectively). However, on choice test, a weak correlation was found between fruit diameter and the number of eggs laid (R^2=0.182 in fresh fruit and 0.031 in fruit-like shape) (Figure 7). In blueberry, high correlation was recorded between fruit diameter and number of eggs laid on no-choice test on fruit-like shape (R^2=0.693), but the correlation was weak in fresh fruit (R^2=0.169). While, on choice test, a high correlation was found in fresh fruit (R^2=0.693) but was weak on fruit-like shape (R^2=0.073).

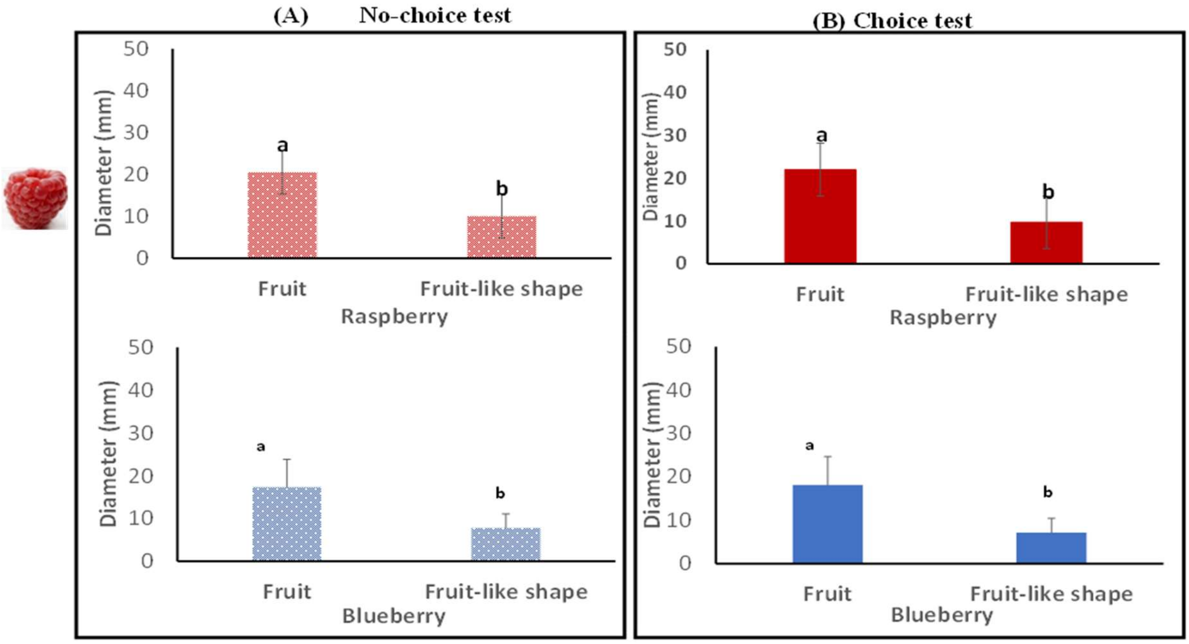

Figure (5): **Fruit diameter (mm)** measurements of fresh fruit vs. fruit-like shape for raspberry and blueberry (A) in no-choice test and (B) choice test. Bars refer to mean+ SE, and different letters over the bars indicate significant differences (Tukey-Kramer HSD, P <0.05).

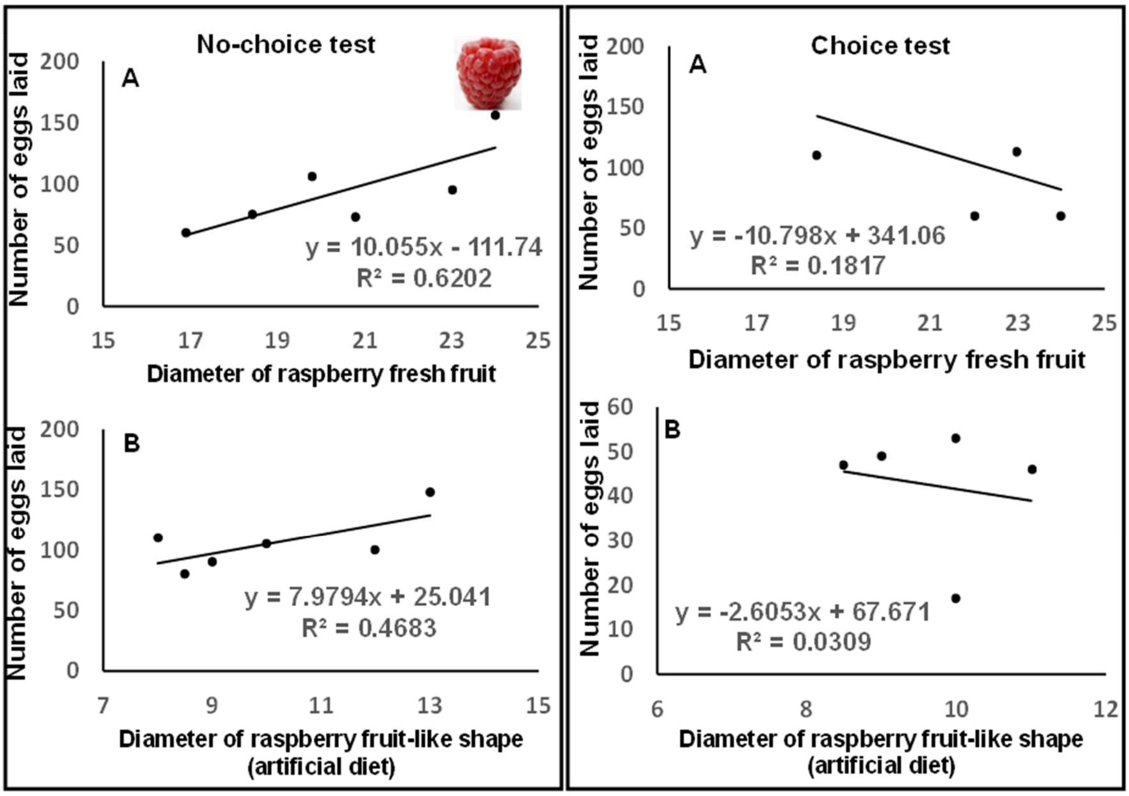

Figure (6): Correlation between diameter of the fruit and number of eggs laid by *D. suzukii* females on no-choice and choice tests on (A) raspberry fresh fruit and (B) raspberry fruit-like shapes artificial diets.

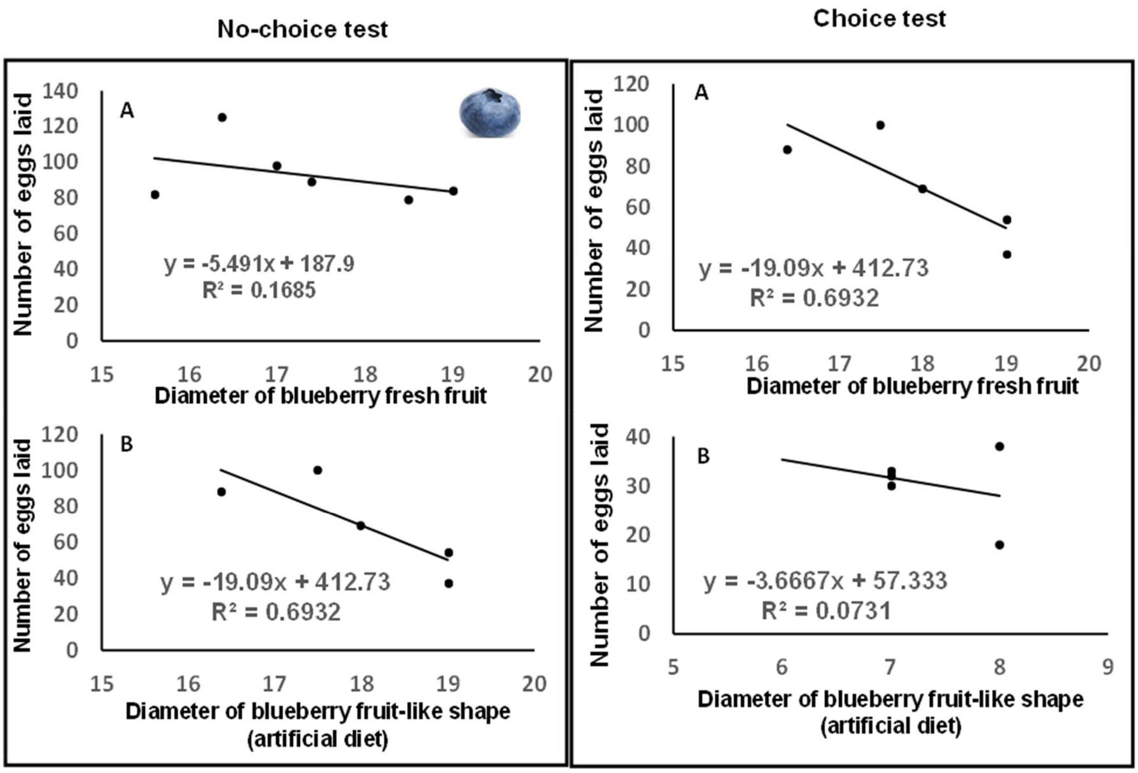

Figure (7): Correlation between diameter of the fruit and number of eggs laid by *D. suzukii* females on no-choice and choice tests on (A): blueberry fresh fruits, (B): blueberry fruit-like shapes artificial diets.

3. Development of *D. suzukii* immature and mature stages on fresh and fruit-like shape fruits

Drosophila suzukii egg laid by females, number of larvae, pupae, total emerged adults and their sex and development time for larvae, pupae and adults were determined and presented in (**Table 1**).

In no-choice test, the mean number of eggs laid was not significantly differed among raspberry treatments and blueberry treatments (ANOVA two-way, DF=1, F=0.18, P=0.673). The number of eggs laid ranged between 92.8-105.5 eggs for raspberry and blueberry treatments. The mean number of *D. suzukii* larvae significantly differed between hosts (ANOVA two-way, DF=1, F=17.9, P=0.0004). The highest number of larvae were recorded on fresh fruit of raspberry (92.2 larvae), while the lowest number was observed on fresh fruit of blueberry (47.5 larvae). Fruit-like shape of raspberry did not significantly differ from fresh fruit of raspberry. However, raspberry and blueberry hosts showed a significant difference between them in number of pupae (ANOVA two-way, DF=1, F=24.4, P< 0.0001). Fresh fruit of raspberry had higher number of pupae (82.7 pupae) followed fruit-like shape of raspberry (73.3 pupae) compared to fruit-like shape and fresh fruit of blueberry (33.3 and 38.5 pupae, respectively). *Drosophila suzukii* emerged females showed a significant difference between hosts (ANOVA two-way, DF=1, F=69.4, P< 0.0001). Fresh fruit and fruit-like shape of raspberry had higher number of females (49.5 and 41.2 females, respectively) than fresh fruit and fruit-like shape of blueberry (11.0 and 9.8 females, respectively). Also, the number of emerged males was differed between raspberry and blueberry hosts (ANOVA two-way, DF=1, F=26.5, P< 0.0001). The highest number of males were observed on fresh fruit of raspberry at 23.5 males, while the lowest was detected on fruit-like shape of blueberry <5.0 males. The number of males on fruit-like shape of raspberry had 18.0 males and did not significantly differ from fresh fruit of both raspberry and blueberry. The mean number of total emerged adults was significantly differed between hosts (ANOVA two-way, DF=1, F=54.2, P< 0.0001). More total number of adults were observed on fresh fruits of raspberry (73.0 adults) followed by fruit-like shapes of raspberry (59.2 adults) than blueberry fresh fruits (17.0 adults) and fruit-like shape fruits (14.0 adults). *Drosophila suzukii* larvae had 1.6 day longer development time on fruit-like shapes of blueberry than fresh fruits of both raspberry and blueberry (3.1 days). Otherwise on fruit-like shape of blueberry, the pupae took 0.7, 1.9 and 3.0 days longer compared to fruit-like shape of raspberry, fresh fruit of blueberry and raspberry, respectively. Additionally, the pupal development time was 1 day longer on fresh fruits of blueberry than raspberry fresh fruit. The adult development time on fruit-like shape of blueberry took 2.5 and 4.5 days longer than fruit-like shape of raspberry, fresh fruit of blueberry and raspberry, respectively.

Results showed that the number of eggs laid were different among treatments (ANOVA two-way, DF=1, F=13.9., P=0.0018). *Drosophila suzukii* females had significantly higher number of eggs laid on fresh fruits of raspberry (107.4 eggs) compared to fruit-like shapes of blueberry and raspberry (30.2

and 42.4 eggs, respectively) on choice test.

In addition, significant differences were detected between hosts treatments (ANOVA two-way, DF=1, F=10.1, P=0.0059) and between treatments (ANOVA two-way, DF=1, F=14.5, P= 0.0015). The greatest number of larvae were observed on fresh fruit of raspberry at 99.8 larvae than other treatments which ranged between 13.6-40.4 larvae. Similar results were noticed for *D. suzukii* pupae, where the number of recorded pupae was significantly differed between hosts (ANOVA two-way, DF=1, F=16.7, P=0.0009) and between treatments (ANOVA two-way, DF=1, F=13.0, P=0.0023) and in the interaction between host and treatment (ANOVA two-way, DF=1, F=6.1, P=0.0247). The highest number of pupae were observed on fresh fruit of raspberry (88.8 pupae) compared to fruit-like shapes of raspberry and blueberry and fresh fruit of blueberry (<25 pupae for these treatments). However, the fruit-like shape of blueberry had the lowest number of pupae (7.2 pupae), but not significantly differed from either fresh fruit of blueberry or fruit-like shape of raspberry. The number of emerged females of *D. suzukii* significantly differed between hosts (ANOVA two-way, DF=1, F=23.8, P=0.0002) and between treatments (ANOVA two-way, DF=1, F=15.4, P=0.0012) and in the interaction between host and treatment (ANOVA two-way, DF=1, F=10.4, P=0.0052). The highest number of females was noticed on fresh fruit of raspberry at 56.8 females, while was <15 females on the rest of treatments. Likewise, results were recorded for *D. suzukii* emerged males, where the number of males was greatly differed hosts (ANOVA two-way, DF=1, F=12.8, P=0.0025) and between treatments (ANOVA two-way, DF=1, F=7.3, P=0.0158) and in the interaction between host and treatment (ANOVA two-way, DF=1, F=5.1, P=0.0383). Fresh fruits of raspberry showed the highest number of males at 24.0 males compared to other treatments which did not exceed 6.0 males. Additionally, the total number of emerged *D. suzukii* adults between hosts (ANOVA two-way, DF=1, F=19.9, P=0.0004) and between treatments (ANOVA two-way, DF=1, F=12.4, P=0.0029) and in the interaction between host and treatment (ANOVA two-way, DF=1, F=8.5, P=0.0103). The highest number of larvae and pupae on the fresh fruit of raspberry resulted in the highest number of total emerged adults (80.8 adults) than fruit-like shape of raspberry and both blueberry fresh fruits and fruit-like shape (19.4, 10.5 and 4.6 adults). The larval development time also significantly differed between hosts (ANOVA two-way, DF=1, F=26.0, P<0.0001) and between treatments (ANOVA two-way, DF=1, F=20.5, P=0.0002). Where the larvae took 3.1 days to complete its development on fresh fruit of raspberry, however the development time for larvae elongated 2 days on fruit-like shape of blueberry. While no differences were found in larval development time between fresh fruit of blueberry and fruit-like shape of raspberry at 4.0 days. On the other hand, the pupae were significantly differed between hosts (ANOVA two-way, DF=1, F=29.2, P<0.0001) and between treatments (ANOVA two-way, DF=1, F=93.0, P<0.0001) in their development time. Fresh fruits of raspberry showed the shortest development time (7.7 days), while pupae need 3.4 days longer to complete their development on fruit-like shape of blueberry. In fresh fruit of blueberry, pupae took 9.0 days for their development which significantly shorter than pupal development time in fruit-like shape of raspberry (10.0 days). Similar results were recorded for adult development time, where the adult development time significantly differed between hosts (ANOVA two-way, DF=1, F=240.6, P<0.0001) and between treatments (ANOVA two-way, DF=1, F=204.4, P<0.0001). *Drosophila suzukii* adults took 12.1 days to complete their development on fresh fruit of raspberry, otherwise, the adult development time was 5.1 days longer on fruit-like shape of blueberry. Fresh fruit of raspberry and fruit-like shape of blueberry showed the same adult development time (14.5 and 14.7 days).

Table (1): Mean ± SE number of *D. suzukii* immature (larvae and pupae), mature stages (males, females, and total emerged adults) and development times (larval, pupal and adult) measured in raspberry and blueberry fresh and fruit-like shape fruits on no-choice and choice tests.

Host	Treatments	No. of eggs	No. of larvae	No. of pupae	No. of females	No. of males	No. of total emerged adults	Larval develop-ment time	Pupal developm-ent time	Adult Develo-pment time
No-choice test*										
Raspberry	Fresh fruit	94.2±14.1 a	92.2±13.8 a	82.7±14.6 a	49.5±7.4 a	23.5±4.9 a	73.0±11.9 a	3.1±0.03 c	7.4±0.08 d	12.0±0.1 c
	Fruit-like shape	105.5±9.6 a	83.7±5.6 ab	73.3±4.0 a	41.2±3.0 a	18.0±2.7 ab	59.2±5.0 a	3.6±0.1 b	9.7±0.1 b	14.0±0.3 b
Blueberry	Fresh fruit	92.8±7.0 a	47.5±3.2 c	38.5±4.2 b	11.0±1.9 b	6.0±1.8 bc	17.0±3.5 b	3.1±0.06 c	8.5±0.2 c	14.2±0.1 b
	Fruit-like shape	95.3±9.4 a	54.7±8.4 bc	33.3±6.7 b	9.8±1.8 b	4.2±1.4 c	14.0±3.2 b	4.7±0.2 a	10.4±0.2 a	16.5±0.3 a
Choice test**										
Raspberry	Fresh fruit	107.4±22.4 a	99.8±21.1 a	88.8±18.5 a	56.8±10.7 a	24.0±6.4 a	80.8±16.8 a	3.1±0.04 c	7.7±0.2 d	12.1±0.1 c
	Fruit-like shape	42.4±5.9 b	32.6±5.6 b	24.2±5.0 b	13.4±2.9 b	6.0±1.8 b	19.4±4.4 b	3.9±0.08 b	10.0±0.2 b	14.5±0.3 b
Blueberry	Fresh fruit	69.6±10.3 ab	40.4±5.6 b	19.2±2.8 b	7.5±1.0 b	3.0±0.7 b	10.5±1.4 b	4.0±0.4 b	9.0±0.2 c	14.7±0.2 b
	Fruit-like shape	30.2±3.0 b	13.6±1.6 b	7.2±0.8 b	3.4±0.5 b	1.2±0.2 b	4.6±0.5 b	5.0±0.2 a	11.1±0.2 a	17.0±0.1 a

*Columns followed by the same letter are not significantly differed from each other at 0.05 Probability.
** Columns followed by the same letter are not significantly differed from each other at 0.05 Probability..

Discussion

The oviposition selection of *D. suzukii* is crucial as it defines the environment for the next generation of larvae. Females prefer oviposition choices that reflected the differences in larval nutritional performance and macronutrient preferences. The preference–performance hypothesis predicts that insect females maximize their fitness by using host plants which are associated with high larval performance (Wertheim *et al*., 2002). However, studies with several insect species have failed to find a positive correlation between oviposition preference and larval performance; the nature of this correlation depends often on the degree of host specialization and seems to decrease in polyphagous insect species (Jaenike, 1990; Mayhew, 1997; Friberg *et al*., 2015). Our results indicated that *D. suzukii* females prefer to lay more eggs on fresh fruit than fruit-like shapes made of artificial diet especially for raspberry host in choice test. However, the number of eggs laid did not significantly differ between fresh fruit and fruit-like shape of raspberry in the no-choice test. Our results suggest that when *D. suzukii* have an opportunity to choose an oviposition site, it prefers fresh fruit for its oviposition, but if there is no chance such as no-choice test, it lays egg in whatever it found. Our findings in agree with (Cai *et al.,* 2019) who mentioned that when the preferred and more suitable host fruits are not available, *D. suzukii* may readily utilize the abundant blueberry and grape crops. Also, *D. suzukii* can oviposit in a wide range of commercial, wild, and ornamental fruits; when other food sources are not available, they can feed on tree sap, honey dew, and host blossoms (Tochen *et al.,* 2016b; Little *et al.,2*017).

A higher number of eggs laid was observed on fresh fruit of raspberry (107.4 egg) in choice test compared to fruit-like shape of both raspberry and blueberry which recorded 42.4 and 30.2 egg, respectively. These results could be interpreted that *D. suzukii* may follow plant volatiles as cues to locate hosts (Bruce and Pickett, 2011), and it is well accepted that fruit volatiles play an important role in the orientation (Faucher *et al.,* 2013) and oviposition activity of *Drosophila* spp. (Stensmyr *et al*., 2012). Furthermore, (Cai *et al.,* 2019) declared that when *D. suzukii* given a choice between fruit and a no odor control, females are attracted to odors for ovipositing from all the intact fruit tested, reconfirming that fruit volatiles are important in host selection by *D. suzukii* and that this species can attack a broad variety of hosts (Bellamy *et al*., 2013). Our results agree with (Hauser, 2011 and Poyet *et al.,* 2014) who mentioned that *Drosophila suzukii* favors fresh and ripening fruits for oviposition. Bernays and Chapman, (2007) declared that the host acceptance in generalist species is in part constrained by deterrent compounds found in plants. The presence of conspecific deterrent signals left behind by individuals on plant tissue may act to further narrow host choice by guiding oviposition site selection.

On the other hand, previous research indicated that host, temperature, and humidity influence the reproductive output of *D. suzukii* (Hamby *et al.,* 2016), suggesting that the oviposition preferences among fruit cultures may also be affected by factors other than odor cues at close range, such as fruit toughness, color, shape, texture and size, pH, or indumenta (Lee *et al*., 2011a; Poyet *et al.,* 2014; Hamby *et al.,* 2016). Previous studies suggested that *D. suzukii* adult cannot survive when they only have access to reproductive sites (i.e., intact fruits), but if there are larger wounds on the exocarp, flies obtain nutrients that are essential for survival (Plantamp *et al.,* 2017). Cai *et al*., (2017) speculated that *D. suzukii* females target damaged fruit for both oviposition and adult feeding and the emitted volatiles from fruit flesh are often chemically distinct from the volatiles emitted from the fruit surface. Additionally, raspberry fresh fruit is soft and easy for *D. suzukii* female for penetration and egg laid compared to fresh fruit of blueberry and artificial diet which are firmer and thicker, respectively.

According to several previous studies, the firmness of the fruits may affect the efficacy of oviposition by *D. suzukii* females (Kawase and Uchino 2005, Lee *et al*. 2011a). Blueberries fruit is much firmer and harder for *D. suzkii* oviposition than raspberries fruit. Kinjo *et al.,* (2013) mentioned that more eggs tended to be laid in berries of blueberry cultivars possessing softer fruits than in those having firmer fruits. Also, he edited that one female was allowed to oviposit on blueberry fruits with different firmness in choice test, showed that softer fruits were more vulnerable to *D. suzukii* females than firmer fruits. However, Silva-Soare *et al.,* (2017) and Guo *et al.,* (2020) stated that *Drosophila suzukii* can lay eggs into both hard and soft agarose gel substrates. Otherwise, the diameter of fresh fruit for both raspberries and blueberries is larger than fruit-like shapes of raspberry and blueberry which may be a good surface area for *D. suzukii* oviposition. This suggestion is in contrast with (Akutsu and Matsuo, 2022) who found that *D. suzukii* laid more eggs on the artificial diet with smaller surface radii (4.8 and 5.7 mm) compared with larger radii (7.7 and 9.6 mm). Our results indicate that fresh fruit of raspberry always had the highest number of *D. suzukii* larvae, pupae and emerged adults compared to fresh fruit and fruit-like shapes of blueberry and raspberry. While, in the no-choice test, there is no significant difference was found between fresh fruit and fruit-like shape of raspberry in the number of immature and immature stages of *D. suzukii*. Previous studies on the behavior of *D. suzukii* oviposition host preference revealed that fresh fruit and artificial diet of raspberry are the most preferred hosts for female oviposition followed by blackberries, blueberries, and strawberries (Aly, 2018 and Bellamy *et al.,* 2013). Moreover, larvae, pupae and adults developed faster in fresh fruit of raspberry compared to fresh fruit of blueberry and fruit-like shapes of blueberry and raspberry, except for larval development in no-choice test, where the larvae had a similar development time on fresh fruit of both raspberry and blueberry. These results suggested that raspberry fresh fruit is suitable host for *D. suzukii* development (Aly, 2018). Hardin *et al.,* (2015) stated that *D. suzukii* larvae developed faster in raspberry suggesting that sufficient nutrients were acquired in raspberry, so as not to hinder pupal development time. Otherwise, the development time elongated for larvae (0.5-1.9 days), pupae (1.1-3.4 days), and adults (2-4.9 days) on fresh fruit of blueberry and artificial diets (fruit-like shapes) of raspberry and blueberry. These results suggested that blueberry fruits and artificial diets are less suitable for development of immature stages of *D. suzukii*. Forney, (2011) stated that blueberries are the least fragrant than raspberries and strawberries fruits, which may explain their poor attractiveness when other fruits are present. Also, he mentioned that blueberry firmness increased for a given cultivar and ripeness stage, fewer eggs were laid and fewer *D. suzukii* developed from those berries. This follows the expectation that firmer fruit is probably harder for *D. suzukii* to utilize. As pH increased, more eggs were laid and more *D. suzukii* developed, suggesting that this fly may fare better in less acidic blueberries.

Furthermore, the artificial diet elongated the development time for all *D. suzukii* stages, this may refer to the presence of microbes on the diet delayed their development. Our findings agree with (Sato *et al.,* 2021) who stated that egg-laying decisions in *Drosophila* are strongly influenced by the presence of microbial growth, suggesting that they are sensitive to microbe-derived cues. They also mentioned that the presence of microbes on the oviposition substrate enhances egg laying of *Drosophila melanogaster and Drosophila biarmipes* but discourages that of *D. suzukii*.

Conclusion

It could be concluded that fresh fruit is favorable to *D. suzukii* females for oviposition than fruit-like shapes made of artificial diets for either raspberry or blueberry. Additionally, the highest number of larvae, pupae, females, males, and total number of emerged adults were recorded on fresh fruit of raspberry. *Drosophila suzukii* larvae, pupae and adults develop faster on fresh fruit of raspberry compared to blueberry fresh fruit and fruit-like shapes of blueberry and raspberry.

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