



Evaluation of host susceptibility, preference and offspring performance of *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) on different hosts

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Abstract

The melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) is a quarantine insect pest worldwide and affects the vegetable quality through its direct feeding and indirectly by passing way for secondary pathogens. This study investigated the host susceptibility, preference and offspring performance of *Z. cucurbitae* under the laboratory conditions. Different vegetable hosts i.e., brinjal (*Solanum melongena* L.), bitter melon (*Momordica charantia* L.), zucchini (*Cucurbita pepo* L.), bottle gourd (*Lagenaria siceraria* [Molina] Standley) and cucumber (*Cucumis sativus* L.) were tested under no choice and free choice tests. Results showed that *C. sativus* and *C. pepo* have highest number of visits/host and oviposition puncture/host. *C. sativus* showed highest pupal recovery and pupal weight in both only choice and free choice test. While, highest percentage of emergence and female offsprings were observed in *C. pepo* under only choice and free choice scenarios. Furthermore, maximum deformities in progeny were observed in case of *L. siceraria* under both test case scenarios. The current study provides exploratory support that fruit flies respond differently to host species that co-exists in field under choice and no choice test. Further, hosts of advantage to fruit flies are adopted more. The host and choice preference of fruit flies have the influence on the pest management strategies for the vegetable crops.

Keywords *Cucumis sativus* · *Cucurbita pepo* · Free choice test · Fruit fly · Recovered pupae · *Zeugodacus cucurbitae*

Introduction

The melon fruit fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) is distributed throughout tropical, subtropical countries (Fletcher 1987) and considered as federal quarantine pest in Asia and Hawaii (Allwood et al. 1999). Its damage can cause 30 to 100% losses in vegetables depending on environmental conditions and crop susceptibility (Kabir et al. 1991; Gupta and Verma 1992; Dhillon et al. 2005). In Pakistan, where adequate management practices are not being implemented in cucurbits, losses have been estimated at 24%

(Stonehouse et al. 1998). Female *Z. cucurbitae* punctures the soft skin of host with the ovipositor and lays eggs below fruit exocarp (Mir et al. 2014). The actual damage is inflicted by the feeding action of the larval stage which consumes fruit pulp and by burrows inside the fruit, thereby creating entry point for fungal infections. The oviposition marks and presence of larvae as well as secondary infections lower the marketability and value of fruits and vegetables either for local or export markets (Ekesi and Billah 2006; Chen and Ye 2007).

For developing successful management strategies, the knowledge regarding biology, different life stages and host preference of certain insect pests is essential (Mir et al. 2014). The preference- performance hypothesis plays an important role in host use patterns of insects (Khan and Tahira-Binte-Rashid 2011). In documented case studies for insect pests, host performance may vary and could be vital for the host shifting. About 25–40% insects are host specific species (Bush and Butlin 2004). Fruit flies behavior differs in different host plants which influence the selection pressure regarding the biological attributes of insects (Moreau et al. 2017).

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Adult female fruit flies are considered to make decisions to oviposit based on the stability of host species for their offspring performance (Kostal 1993; Joachim-Bravo et al. 2001; Fontellas-Brandalha and Zucoloto 2004). In *Bactrocera* flies, host selection behavior may be affected by skin, size, odor, color, fruit shape foliage characteristics (Brévault and Quilici 2007; Jaleel et al. 2018). Moreover, nutritional level of host can affect the offspring development (Balagawi et al. 2005). In addition, different toxins and resins may also affect the development of fruit flies (Seo et al. 1982; Rattanapun et al. 2009). Usually a broad range of hosts co-exist in field conditions during summer season and the majority of which are susceptible to fruit fly infestation. Previous studies reported oviposition preference and offspring performance of fruit flies based on choice and no-choice trials (Muthuthantri and Clarke 2012; Rauf et al. 2013; Rizk et al. 2014 El-Gendy 2017). The findings of the above mentioned studies demonstrated an oviposition preference hierarchy of fruit flies on host tested. Further, these studies provide a possible role of adult preference for host as an important function in differential oviposition. The current study was conducted to gain detail information to determine whether fruit flies have preference if given choose. Such information is important in determining host susceptibility and fruit fly offspring performance. The current study was therefore conducted to determine oviposition preference of *Z. cucurbitae* on different hosts and scenarios Materials and methods.

In the present study, *Z. cucurbitae* adults were collected from field area (35°55'11.36"N, 74°22'47.44"E) in Gilgit and kept at Centre for Agriculture and Bioscience International (CABI), Gilgit, Pakistan (35°55'21.3"N 74°21'42.9"E). The fruit fly culture were kept (for 10 generations before the experiments) in perspex rearing cages (60 × 60 × 48 cm) with a cloth sleeve opening at front with a capacity of 1000 female and male flies (1♀:1♂) and maintained at 28 ± 1 °C; 65 ± 5% RH, and photoperiod of L14: D10.. The adult flies were fed on a water based diet consisting of yeast, banana, egg yolk, vitamin B-complex and sugar (unpublished material) and were provided with water on soaked cotton ad libitum. Adult flies were provided with fresh host for laying their eggs. Later, infested vegetables were removed from cages and kept in plastic containers with sand as pupation medium at bottom and kept till pupation. After pupation, pupae were sieved using mesh screen (80) and kept in jars (15 × 6 × 6 cm) till emergence.

Host plants

Different host plants that co-exist with *Z. cucurbitae* in field at Gilgit including bitter melon (*Momordica charantia* L.), zucchini (*Cucurbita pepo* L.), bottle gourd (*Lagenaria siceraria* [Molina] Standley), cucumber (*Cucumis sativus* L.) and brinjal (*Solanum melongena* L.) were used. The above mentioned hosts were purchased from a local market in Gilgit,

Pakistan. Hosts were selected based on no prior infestation or damage. Five to seven days old *Z. cucurbitae* adults were used for further experimentation while maintaining sex ratio (1:1). Male and female flies were identified according to Drew and Raghu (2002). The adults were provided with the water based diet and water ad libitum. The experiments were conducted at standard laboratory conditions as previously described. Each host preference treatment was replicated thrice for all tests and repeated twice.

Host susceptibility, preference and off spring performance

Choice and no choice test were conducted under laboratory conditions to assess the host susceptibility, preference and offspring performance of *Z. Cucurbitae*. **No choice test** *Z. cucurbitae* pairs (50) (1♀:1♂) were placed in plastic jars (15 × 6 × 6 cm) with ~300 g of host plant material (described above) offered separately and left for 48 h. For number of visits per day hosts were observed for 10 h and each host was watched for 2 min/h (Jaleel et al. 2018). Later, exposed hosts were replaced by new hosts in the jars and infested host and oviposition punctures/hosts was counted. Infested hosts were kept separately in plastic jars and sand substratum offered at bottom for pupation later sieved to separate pupae, followed by recording the number of pupae. Later, pupae were weighted in groups and mean pupal weight was calculated (explained in statistical analysis). The pupae were kept in plastic jars for adult emergence, sex ratio and deformity i.e., wings, abdomen and proboscis.

Choice test

For free choice test, *Z. cucurbitae* pairs (50) (1♀:1♂) were placed in plastic jars (15 × 6 × 6 cm) with hosts (described above) offered simultaneously after determining the weight of each host (~300 g) for egg laying for 48 h. The observation was recorded to investigate efficiency with same parameters as described in “no choice test”.

Statistical analysis

The mean pupal weight (mg) was calculated with aid of following formulae:

$$\text{Pupal Weight (\%)}[\Sigma Y] = \left(\frac{\text{Sum of all pupal group replications}}{\text{no. of observation}} \right) [\Sigma X]$$

The adult flies emergence (%) was calculated using following formulae:

$$\text{Adult emergence (\%)} = \frac{\text{Number of adults emerged}}{\text{Total number of pupae}} \times 100$$

While sex ratio (female) was calculated as by Farooq and Freed (2016) using the following formulae:

$$\text{Sex ratio (female)} = \frac{\text{Number of females emerged}}{\text{Total (male + female)}} \times 100$$

The data on means were subjected to Analysis of Variance (ANOVA) to determine means of different parameters i.e., number of visits per day, oviposition puncture/host, recovered pupae, pupal weight, adult emergence, sex ratio and deformity were separated using Tukey's HSD test value at 0.05% level of significance. All analyses were performed using Statistic 8.1 (McGraw-Hill 2008).

Results

The results regarding host susceptibility and off spring performance attributes i.e., number of visits per day, oviposition puncture/host, number of recovered pupae, pupal weight, adult emergence, deformity and sex ratio of *Z. cucurbitae* under no choice and free choice tests showed significant variations.

Fruit visits

For no choice test, host plant type had a significant effect on number of visits by *Z. cucurbitae* per day ($F_{4,14} = 11.1$, $P = 0.001$) with *C. sativus* being visited more time a day (9.67 ± 0.88) followed by *C. pepo* (8.33 ± 0.33), *M. charantia* (5.33 ± 1.20), *S. melongena* (4.00 ± 0.58), and lastly *L. siceraria* (3.00 ± 0.58) s (Fig. 1a). In addition, host plant type also had a significant effect on the oviposition puncture by female flies ($F_{4,14} = 42.0$, $P = 0.000$) with *C. sativus* had most ovipositional punctures (17.00 ± 1.53) followed by *C. pepo* (15.33 ± 0.88) *S. melongena* (7.33 ± 0.67), *M. charantia* (6.33 ± 0.67) and lastly *L. siceraria* (3.67 ± 0.33) in no choice test (Fig. 1a).

In case of free choice test, similar trend was observed host plant type had a significant effect on number of visits by *Z. cucurbitae* ($F_{4,14} = 44.9$, $P = 0.000$) with *C. sativus* being visited more time (9.33 ± 0.40) followed by *C. pepo* (8.00 ± 0.58) *S. melongena* (2.67 ± 0.33), *M. charantia* (2.67 ± 0.33) and lastly *L. siceraria* (1.33 ± 0.33) (Fig. 1b). In addition, host plant type also had a significant effect on oviposition puncture by female flies ($F_{4,14} = 33.2$, $P = 0.000$) with *C. sativus* had most ovipositional punctures (14.67 ± 1.45) followed by *C. pepo* (11.67 ± 1.20), *M. charantia* (5.00 ± 0.58), *S. melongena* (4.33 ± 0.33) and lastly *L. siceraria* (2.00 ± 0.58) (Fig. 1b).

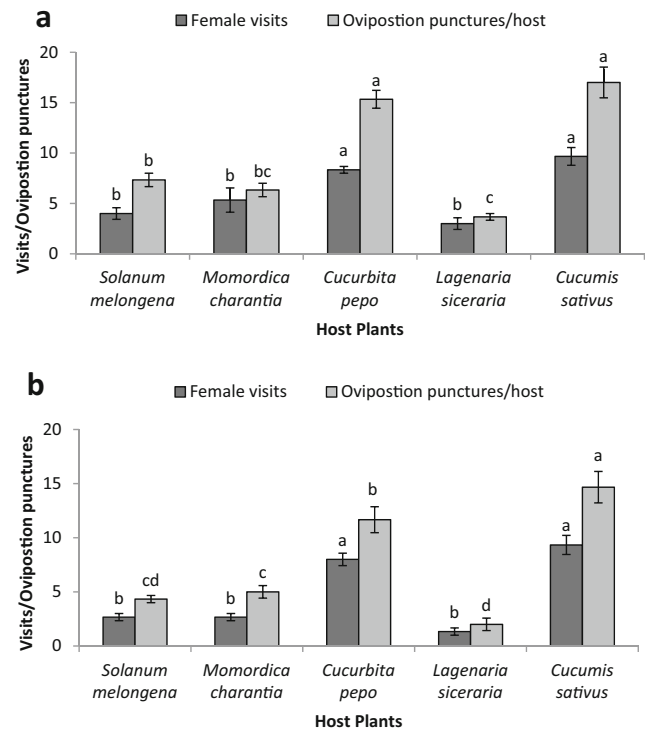


Fig. 1 Number of female visits and oviposition punctures by *Zeugodacus cucurbitae* **a** under no choice and **b** free choice scenarios on different hosts. Means followed by same letters for each parameter are not statistically different; HSD, $P < 0.05$

Recovered pupae

The results of no choice test showed that host plant type had a significant effect on recovered pupae ($F_{4,14} = 94.4$, $P = 0.000$) with *C. sativus* had highest pupal recovery (1003.67 ± 57.85) followed by *C. pepo* (970.0 ± 56.86), *M. charantia* (669.67 ± 15.38), *S. melongena* (320.67 ± 15.07) and lastly *L. siceraria* (187.67 ± 14.68) (Fig. 2a). Similar trend was observed in free choice test as significant effect of host plant was observed on recovered pupae ($F_{4,14} = 64.4$, d.f. = 4,14, $P = 0.000$) with *C. sativus* (450.33 ± 36.67) had highest pupal recovery followed by *C. pepo* (370.0 ± 20.84) *M. charantia* (197.0 ± 19.92), *S. melongena* (112.0 ± 14.04) and *L. siceraria* (28.67 ± 4.49) (Fig. 2a).

Pupal weight

Under only choice test, significant effect of host plant was observed on pupal weight ($F_{4,14} = 84.2$, $P = 0.000$) with *C. sativus* (14.03 ± 0.37) showed maximum pupal weight, followed by *C. pepo* (13.13 ± 0.20), *M. charantia* (10.34 ± 0.1) *S. melongena* (9.32 ± 0.22) while, *L. siceraria* (8.41 ± 0.34) showed the maximum reduction in it (8.41 ± 0.34) (Fig. 2b). Similar trend was observed in free choice test as results regarding pupal weight was significantly influenced by host type ($F_{4,14} = 230.0$, d.f. = 4,14, $P = 0.000$) with *C. sativus* (14.15 ± 0.22) (mg)

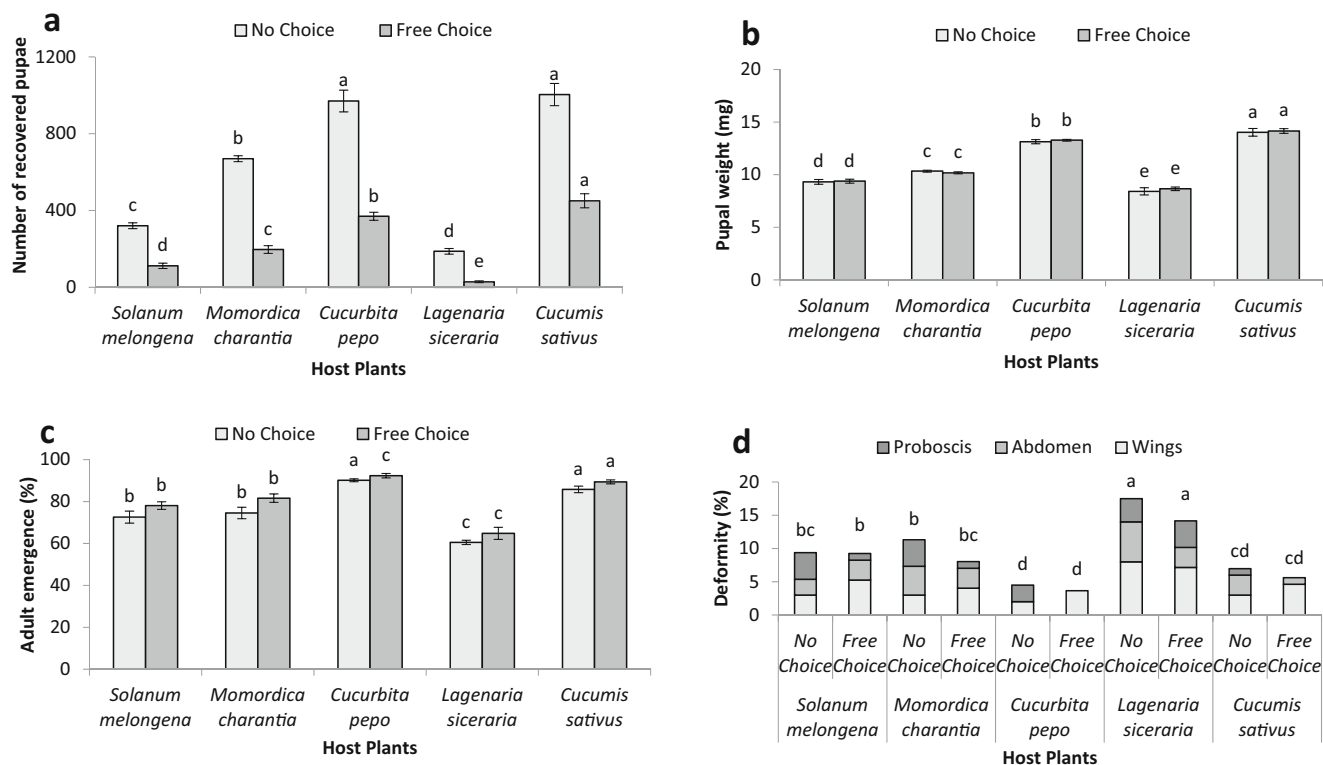


Fig. 2 Effect of host plants on different attributes of *Zeugodacus cucurbitae* under only choice and free choice test, **a** recovered pupae, **b** pupal weight (mg), **c** adult emergence (%) **d** deformity (%). Means

followed by same letters for each column (only choice, free choice test) are not statistically different; HSD, $P < 0.05$

showed maximum pupal weight followed by *C. pepo* (13.28 ± 0.1), *M. charantia* (10.17 ± 0.1) *S. melongena* (9.39 ± 0.18) while, *L. siceraria* (8.66 ± 0.17) showed the significant reduction in it (8.66 ± 0.17) (mg) (Fig. 2b).

Adult emergence

The data regarding the adult emergence under no choice test showed that adult emergence was significantly influenced by host type ($F_{4,14} = 34.6$, d.f. = 4,14, $P = 0.000$) as *C. pepo* (90.15 ± 0.70) had the highest adult emergence followed by *C. sativus* (85.81 ± 1.59), *M. charantia* (74.54 ± 2.74) *S. melongena* (72.60 ± 2.86) and lastly *L. siceraria* (60.52 ± 1.02) (Fig. 2c). While for free choice test, adult emergence was significantly influenced by host plant type ($F_{4,14} = 33.3$, $P = 0.000$) as highest adult emergence was observed in *C. pepo* (92.34 ± 1.07) followed by *C. sativus* (89.39 ± 0.94) *M. charantia* (81.63 ± 1.07) *S. melongena* (78.08 ± 1.98) and lastly *L. siceraria* (64.83 ± 1.07) (Fig. 2c).

Sex ratio

The results regarding sex ratio showed that host plant had significant effect of female percentage under no choice test ($F_{4,14} = 7.36$, d.f. = 4,14, $P = 0.005$) as *C. pepo* had the highest

number of females (52.35 ± 0.38) followed by *C. sativus* (48.75 ± 1.92), *L. siceraria* (44.23 ± 1.41), *M. charantia* (42.98 ± 1.30) and lastly *S. melongena* (41.14 ± 2.60) (Table 1). In case of free choice test, significant effect on host plant type were observed ($F_{4,14} = 7.36$, $P = 0.005$) as *C. pepo* showed the highest number of females (52.92 ± 0.87) followed by *C. sativus* (52.57 ± 1.08), *M. charantia* (47.58 ± 2.14), *S. melongena* (45.47 ± 1.60) and lastly *L. siceraria* (41.19 ± 2.81) (Table 1).

Table 1 Effects of different host species on sex ratio (♀)(%±SE) of *Zeugodacus cucurbitae* under only choice and free choice test

Host Plant	no choice test	Free choice test
<i>Solanum melongena</i>	41.14 ± 2.60c	45.47 ± 1.60bc
<i>Momordica charantia</i>	42.98 ± 1.30c	47.58 ± 2.14ab
<i>Cucurbita pepo</i>	52.35 ± 0.38a	52.92 ± 0.87a
<i>Lagenaria siceraria</i>	44.23 ± 1.41bc	41.19 ± 2.81c
<i>Cucumis sativus</i>	48.75 ± 1.92ab	52.57 ± 1.08a
F value	7.36	7.23
P value	0.005	0.005
HSD-value	5.32	5.80

*Means in columns, followed by same letter are not significantly different according to the Tukey’s HSD test, $P < 0.05$

Deformity

The attributes of emerging flies showed significant variations due to host plant type under no choice test ($F_{4,14} = 25.7$, d.f. = 4, 14, $P = 0.000$) as *L. siceraria* (17.54 ± 0.53) showed the maximum deformed flies (combine attributes) followed by *M. charantia* (11.32 ± 0.77), *S. melongena* (9.38 ± 1.20), *C. sativus* (6.99 ± 1.44) and *C. pepo* ($4.510.61$) (Fig. 2d). Furthermore, a similar trend for deformed new flies was observed in free choice test ($F_{4,14} = 25.4$, $P = 0.000$) as *L. siceraria* (14.16 ± 1.17) showed highest deformed flies followed by *S. melongena* (9.25 ± 0.38), *M. charantia* (8.03 ± 0.71), *C. sativus* (5.61 ± 0.94) and *C. pepo* (3.66 ± 0.50) (Fig. 2d).

Discussion

A significant influence of host plants was observed on the different developmental parameters of the fruit fly. In the current study, *Z. cucurbitae* preferred *C. sativus* and *C. pepo* for oviposition and immature feeding under both no choice and free choice scenarios. The results of number of female visits, oviposition puncture/host and pupal recovery showed higher preference *C. sativus* > *C. pepo* > *M. charantia* > *S. melongena* > *L. siceraria* under only choice and free choice test in that order. Our findings suggest all five tested hosts can be utilized by *Z. cucurbitae* depending upon host availability. Host quality plays a key role on the development of offsprings. Thus both host species and its quality had an influence on the development of immature stages of *Z. cucurbitae*.

The host preference of fruit flies was determined by different attributes including pupal size, adult emergence percentage, sex ratio and deformity percent under host preference studies. (Li-Li et al. 2008; Rauf et al. 2013). In the present study, *C. sativus* and *C. pepo* were most preferred hosts by *Z. cucurbitae* as shown by high pupal recovery under both scenarios. Similar results were reported by Khan and Tahira-Binte-Rashid (2011) where bitter melon and cucumber were the most susceptible host for *B. cucurbitae* as shown by pupal yield recovery. Body sizes of insect being an indicator for insect fitness, large insects are more competitive as frequent mating, high fertility and more dispersion capacity (Navarro-Campos et al. 2011; Thorne et al. 2006). Further, plant species differ in their suitability for serving a food source for insects. In fruit flies host preference is favored by numerous factors including odor, color, shape and size of host (Li-Li et al. 2008; Bruce et al. 2005).

Based on the results of the current study for obtained pupae and adult emergence, it is suggested that *Z. cucurbitae* can use these natural hosts depending upon their availability and also preference of fruit fly. Similar to the current study, Fitt (1986) noted the fruit fly species abundance on different hosts as female flies make more choices as compared to larval specialization. Although many host plants can provide bursting

sustenance of tephritid species, yet host quality administrates the differences in larval specialization and survival rate. The relationship between host preference and offspring performance provides support for the preference-performance hypothesis as female insects will evolve themselves to oviposit more on the hosts which can support their offspring fare better (Akol et al. 2013). Female fruit fly's response toward a host is influenced by olfactory, visual and contact cues such as the color, size, shape and smell of host fruit (Drew et al. 2003; Brévault and Quilici 2007; Khan and Tahira-Binte-Rashid 2011).

The health of pupae directly influenced the adult emergence while pupa is dependent on the larvae. An adult fails to emerge due to poorly developed pupa. Adult deformity increases as per poor host section and reduces adult emergence (Mayhew 1997). The current study showed that *L. siceraria* was proved to be a most un-preferable host by *Z. cucurbitae* signified by the different parameters. The studied vegetables crops showed significant effects on developmental attributes of *Z. cucurbitae*. The difference in egg laying preference, different pupal weight different sex ratio and deformity of adults on different hosts could be due to number of factors e.g. plant phenology, nutritional, microbial contamination, fruit temperature, peel thickness and maturity of fruits (Feder et al. 1997). Host plants differ in the carbon, nitrogen composition and metabolites which interrupt insect oviposition, feeding, growth and development leading to low fitness and low offspring survival (Roitberg and Isman 1992; Haggstrom and Larsson 1995; Awmack and Leather 2002; Gibbs et al. 2006). Previous studies by Hafsi et al. (2016) explained 30% variability in the larval performance of different fruit fly species was due to water, lipid, carbohydrates and fiber contents of hosts. Moreover, a positive correlation of polyphagous species of fruit flies exhibits for carbohydrate, lipid, and fiber contents while it negatively correlates with water content. For fruit fly species associated with cucurbit hosts a positive correlation with water content and negative correlation with carbohydrate and lipid content exists. The results of previous studies (Zucoloto 1987; Joern and Behmer 1997; Lee et al. 2008; Roeder et al. 2014; Nash and Chapman 2014; Hafsi et al. 2016) showed that carbohydrates were important elements of fruit nutritive value for larvae of tephritid. Host specialization cannot be solely predicted by nutritional measurement as it also affected by secondary metabolites and volatile compounds (Bateman 1972; Renwick 2001). Plant secondary metabolites directly or indirectly influence the fecundity of by being toxigenic or reducing nutrient assimilation (Awmack and Leather 2002). In addition, Erbout et al. (2009) reported that fruits containing high alkaloid concentrations re detrimental for polyphagous tephritid larvae.

The previous experiments on other tephritid have shown that flies adapt behaviorally to the host plants phenology. Several studies have also reported cases where female

preference and performance appear uncoupled, or where the relationship is surprisingly weak (Fritz et al. 2000; Faria and Fernandes 2001). Moreover, some hosts were not able to attract flies than others to a greater extent are easier to explain as cuticle proved as a less preferable oviposition substrate (Feng-Ming 1997). The evolutionary and ecological considerations have been proposed to explain apparent mismatches between choice and performance including the fact that the strength of the preference–performance relationship is modified by ecological and / or life-history factors which may have contributed to the observations noted on cucumber in the current studies (Mayhew 2001). All the tested fruit fly species are polyphagous; strictly attacking cucurbit. Within the context selecting for appropriate host, the female flies may encounter several constraints including limitations on the information processing capacity among similar host plant family (Cunningham 2012). The results of current study regarding insect life history on different vegetable hosts might be used for development of management tools against this pest of concern. However, our study did not determine host plant factors that might interrupt with insect infestation and life history attributes. Therefore, further studies are recommended to explore host plant characteristics with respect to environmental conditions. In addition studies regarding behavior, age-stage and two-sex life table parameters for more detailed knowledge is required to achieve a reliable fruit fly management strategy.

Conclusion

In conclusion, these findings provide supporting evidence that *Z. cucurbitae* prefer *C. sativus* and *C. pepo* as hosts. Based on our findings, *L. siceraria* is less preferred by *Z. cucurbitae* thus such knowledge may be important in relaxing quarantine restrictors and requirements for post-harvest treatments especially for less preferred host plants.

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Author contribution MF performed the experiments, analyzed the data wrote the manuscript. SB performed the experiments. SFH and BEB supervised the work, help in data analysis and article writing. FU performed the experiments and aid in the manuscript write up IHS Help in data analysis and article writing.

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Compliance with ethical standards

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

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