

## SPECIES COMPOSITION OF FRUIT FLIES AND THEIR FEEDING PREFERENCES TO CUCURBITACEOUS CROPS IN NEPAL

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### ABSTRACT

Fruit fly species (Diptera: Tephritidae: Dacinae: Dacini) have been reported in Nepal, primarily based on field monitoring and specimen collections from cucurbitaceous crops. Their management is effective if the control measures are specific to the damage causing species. Therefore, the total 365 infested flowers/fruits of bitter gourd, cucumber, sponge gourd and zucchini/pumpkin were collected from eastern-western terai and mid-hills of Nepal from 7 July to 27 November in 2022. Each sample was placed in separate plastic jar till the adult emergence. The adult fruit flies were collected from each jar and identified based on morphological features and standard keys under trinocular microscope. At least one fruit fly species emerged as adult fly from 75% samples (n=273). *Zeugodacus cucurbitae* (Coq.), *Z. tau* (Walker), *Z. scutellaris* (Bezzi) and *Z. diversus* (Coq.) were recorded from 43%, 55%, 25% and 9% of the 273 samples, respectively. Individual flower/fruit sample infested with one, two and three species of fruit flies were 70.3%, 28% and 1.7%, respectively. *Z. tau* was the most abundant species and accounted for 82.2% while *Z. cucurbitae* accounted 15.6% of the total emerged adults (7799). *Z. cucurbitae* and *Z. tau* emerged from flowers and fruits while *Z. scutellaris* and *Z. diversus* largely emerged from flowers. Considering the species composition within genus *Zeugodacus*, the management strategies should target the damage causing species in field.

**Key words :** *Bactrocera*, dacini, tephritidae, vegetables, *Zeugodacus*

### INTRODUCTION

Cucurbits include cucumber, pumpkin, squash, bitter gourd, sponge gourd, bottle gourd, pointed gourd etc. belonging to the Cucurbitaceae family. In 2021/2022, the cucurbits spread in 58,114 ha of land in Nepal with the average productivity of 12.39 mt/ha (MoALD, 2023). The low productivity is attributed to infestation by fruit flies (Diptera: Tephritidae). Among various problems, fruit flies have been recognized as most serious pests as they cause heavy damage to the fruits of cucurbits. In addition, fruit fly infestation symptoms cause serious impact on its consumers' preference. Though several management practices are being adopted, the damage due to fruit flies is increasing (Sapkota et al., 2009). Their population are distributed worldwide in tropical, subtropical and temperate regions. Over 4700 recognized species of family Tephritidae, very few fruit fly species cause serious economic losses in cucurbits. Most species belong to three genera, *Zeugodacus*, *Bactrocera* and *Dacus* of the tribe Dacini of the sub-family Dacinae (Vasuda et al., 2019). The fruit fly species: *Z. cucurbitae*, *Z. tau*, *Z. diversus*, *Z. cilifer*, *Z. scutellaris*, *B. nigrofemoralis*, *D. longicornis* and *D.*

*ciliatus* have been reported to infest cucurbits (Nair et al., 2022). Among them, *Z. diversus*, *Z. cilifer*, *Z. caudatus* and *Z. scutellaris* caused damage in cucurbit flowers only (Prabhakar et al., 2007; Vasuda et al., 2019) while others *Z. cucurbitae* and *Z. tau* infest flowers and fruits of cucurbits (Nair et al., 2017). Mostly *Zeugodacus* spp. are polyphagous and some of them have repeatedly invaded even in non-native ranges (Vargas et al., 2015). Two fruit fly species; *Z. cucurbitae* and *Z. tau* are commonly recorded in the cucurbit ecosystem in diverse growing conditions (Sawai et al., 2019).

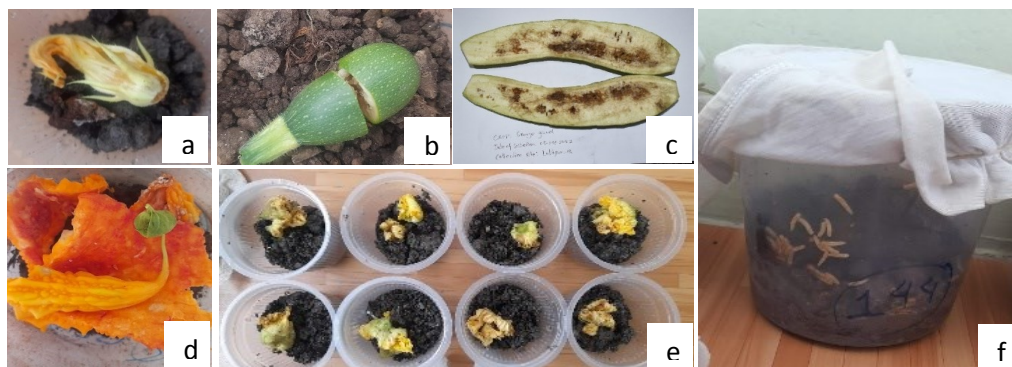
The extent of losses varies depending on the cucurbit crop, variety, season and locality. In Reunion Island, two species *Z. cucurbitae* and *D. ciliatus* were reported from infested bitter gourd and Ivory gourd (Vayssieres et al., 2008). In Padang, Indonesia, *Z. cucurbitae* and two *Bactrocera* spp. found in four vegetable crops with the highest infestation in ridge gourd and the highest diversity of fruit fly species in bitter gourd (Budiyanti et al., 2019). Saeed et al. (2022) reported sponge gourd as preferred host for *Z. cucurbitae* compared to bitter gourd and pumpkin. Fruit flies' preference to the host for oviposition is primarily influenced by the color, smell, taste, and texture of the fruit, as well as the nutritive value of the fruit, which serves as a source of energy and sustenance throughout their development toward adulthood (Shelly, 2000).

In Nepal, twenty-six fruit fly species were reported by Leblanc et al. (2019) while Sapkota et al. (2024) reported 27 fruit fly species including *D. (Callantra) nepalensis* (Hardy). Adhikari (2024) reported 30 fruit fly species including *B. tsuneonis* (Miyake) (later recognized as *B. minax* (Enderlein)) but *D. (Callantra) nepalensis* was not included in this list. These species are largely recognized from specimen collection and field monitoring, and only three fruit fly species: *Z. tau*, *Z. cucurbitae* and *B. minax* were reported to be from infested fruits. The identification of actual infesting fruit fly species from infested flower/fruit have not been investigated exhaustively in Nepal. Since the cucurbits are major hosts and the effective management of this pest is crucial with respect to the infestation causing fruit fly species. So, this study aimed to answer the composition and identification of fruit fly species from infested flowers/fruits of cucurbitaceous crops in Nepal.

## **MATERIALS AND METHODS**

### **Sample and sampling sites**

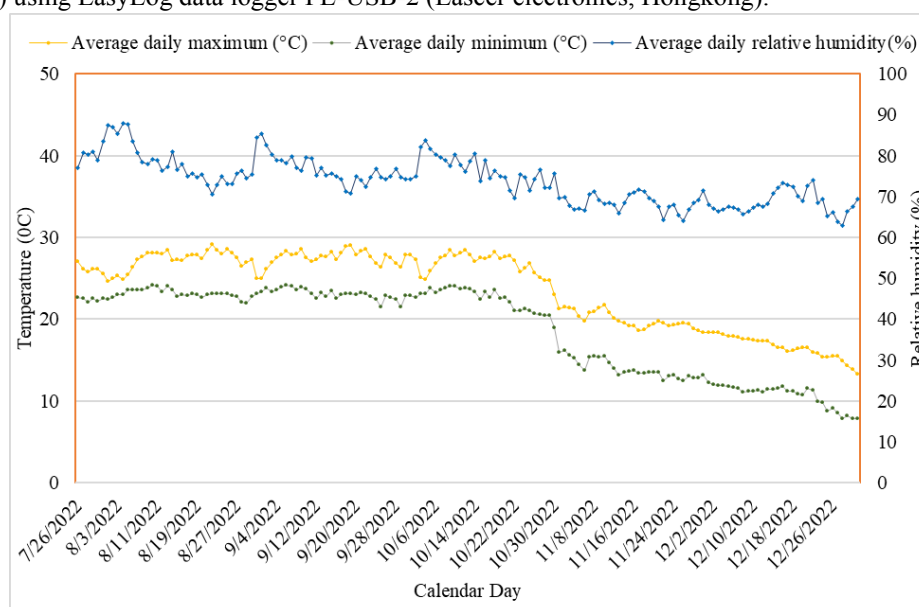
Samples were collected on the basis of infestation in flowers and fruits of cucurbits from six geographically isolated locations; viz., Rupandehi (27°35'N, 83°26'E), Chitwan (27°34' N, 84°30' "E), Mahottari (26°52'N, 85°49'E), Kathmandu (27°42', 85°18'E), Sindhuli (27°10'N, 85°56'E) and Palpa (27°49'N, 83°38'E). These sites represent major cucurbit growing areas in the eastern, central and western part of Nepal. Samples of cucurbit crops (cucumber, zucchini/pumpkin, bitter gourd, and sponge gourd) were collected from respective areas based on their availability during summer-rainy and autumn-winter seasons in 2022. The flowers/fruits collected were packed individually in zip plastic bag and transported for rearing in laboratory. Each sample was characterized based on infestation (puncture noticed, partly damaged and severely damaged), growth stages of host (male flowers, post set fruits with or without female flower, marketable size fruit and matured fruit), location and date of collection (Fig. 1). A total of 365 samples were collected throughout the study period. Continuous sampling of infested flowers / fruits was only done in Kathmandu Valley whereas field infested fruits were collected from other locations during field visits in summer-rainy and autumn-winter seasons.



**Fig. 1.** Different stages of infested cucurbit samples (a. squash flower; b. post set fruit of squash; c. marketable size fruit of sponge gourd; d. matured fruit of bitter gourd; e. sponge gourd flowers; and f. rearing container with maggots).

### Rearing and identification of fruit flies

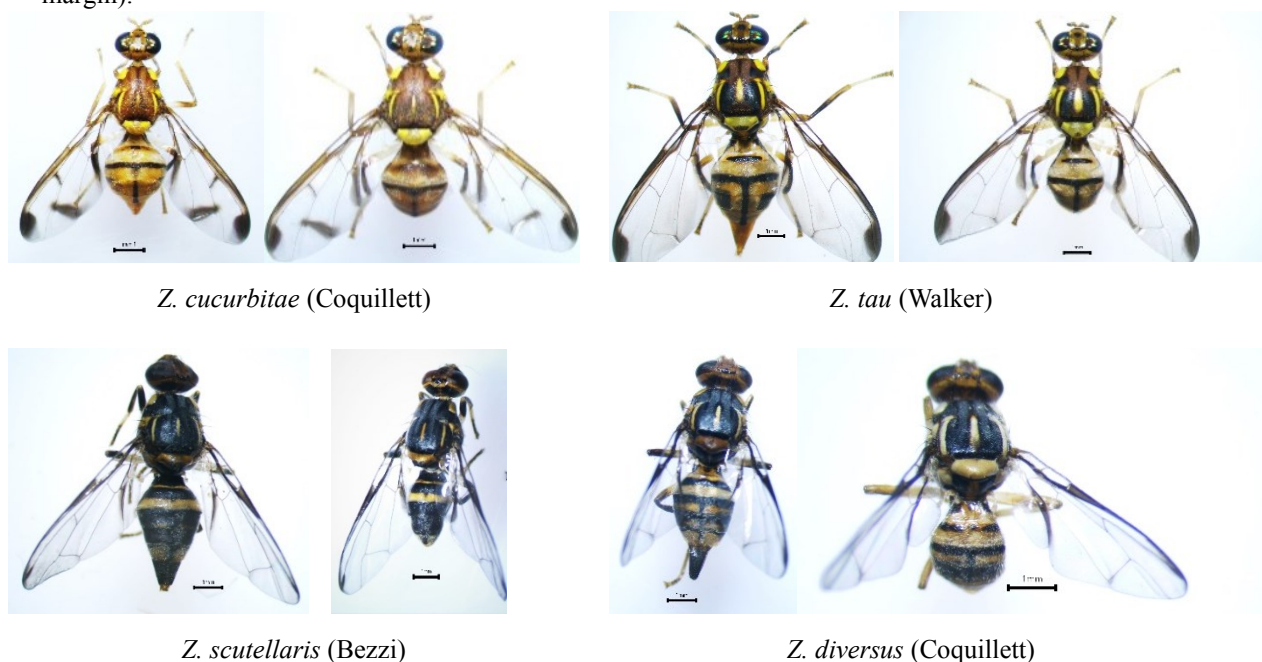
The rearing was conducted in room condition of Kirtipur, Kathmandu. Infested individual flower or fruit was placed in one litre plastic container having 1/3rd of sandy soil to allow maggots for pupation (Fig. 1f). The container was covered with muslin cloth and tightly tied with rubber band. Each container was sprayed with water in every two days interval to maintain moisture. The room temperature and relative humidity were monitored throughout the study period in every hour interval (Fig. 2) using EasyLog data logger FL-USB-2 (Lascor electronics, Hongkong).



**Fig. 2.** Average daily maximum and minimum temperatures (°C), and relative humidity (%) in the laboratory room, July to December, 2022.

The adult fruit flies emerged from each sample were trapped, counted and recorded male and female separately. Each fruit fly was magnified under trinocular stereo microscope (BS 3040T, Bestscope,

China) and identification was done based on the morphological key suggested in the guide book of Australian hand book (Plant Health Australia, 2018) and the guidelines given by David & Ramani (2011), Prabhakar et al. (2012), Nair et al. (2018) and David et al. (2024). The genus *Zeugodacus* was recognized considering the morphological keys: shallow emargination of sternite 5 in males, posterior lobe of surstylus 5–6× longer than anterior lobe, glans of phallus with patterned acrophallus, except some cases, medial postsutural vitta present along with lateral vitta. Four *Zeugodacus* species (Fig. 3) were identified with the key characteristics of *Z. cucurbitae* (wing with radial-medial band and subapical band); *Z. tau* (face fulvous with a pair of circular to oval black spots, wing costal band overlapping vein  $R_{2+3}$  and expanded into an apical spot, scutum black with large areas of red-brown, two pair of scutellar setae); *Z. diversus* (scutum with medial postsutural vitta narrowed at both ends, face with a transverse line in female and fulvous in male, pecten absent) and *Z. scutellaris* (scutellum yellow with an apical black spot, notopleuron black, face fulvous with black markings in lower facial margin).



**Fig. 3.** Fruit fly (female and male) species emerged from infested flower/fruit of cucurbits, 2022.

### Data collection and statistical analysis

Data were calculated to present the average fruit flies emerged per sample with respect to crops, growth stages of host, damage intensity in host, seasons (summer-rainy vs autumn-winter), and sex ratio (male: female). The data were subjected to descriptive analysis and chi-square test. Individual specimen of each sample was identified to know the species diversity within the sample. Comparison was made to compare the sample collected locations in terms of species diversity. The number of days to emerged adult flies was also recorded to compare between seasons and growth stages of hosts.

## RESULTS AND DISCUSSION

### Host characteristics and fruit fly abundance

Adult fruit flies emerged from 75% of the total samples (i.e. 365). One of the potential reasons of failure of fruit fly emergence in 25% sampled might be due to long distance transportation. Recovery of larvae to pupae and pupae to adult varied depending on adequate nutrition of the larvae and atmospheric condition which cause transformation failed to some development stage of fruit flies. In a rearing experiment, temperature plays an important role on pupal development (Hollingsworth et al., 1997) and about 2% pupae failed to develop into adult flies in cucurbits (Salmah et al., 2017). Dominguez (1999) also reported the recovery and transformation of larva to pupae and pupae to adults were 80-90% and 75-95%, respectively in a controlled condition.

The number of adult fruit fly emergence ranged 13-51 per sample depending on cucurbit crops and varied significantly ( $\chi^2$   $p \leq 0.05$ ) (Table 1). The maximum fruit fly number per sample was collected from the marketable size of sponge gourd. Saeed et al. (2022) reported that the sponge gourd as the most preferred host of *Z. cucurbitae* while the *Z. tau* preferred host was pumpkin (Karnjanaungkool & Julsirikul, 2021). The physical and chemical properties of the plant also influence the fruit fly behavior, host preferences, and their biology (Dhillon et al., 2005, Darshanee et al., 2017).

In respect to the damage severity, significantly ( $\chi^2$   $p \leq 0.05$ ) lower number of fruit flies emerged from puncture noticed samples as compared to partly and severely damaged fruits/flowers of cucurbits. Similarly, few number of fruit flies emerged from male flower as compared to fruits. Higher number of fruit flies emerged from samples collected during summer-rainy season (31/sample) than in autumn-winter (17/sample). Seasonal variations of the weather factors play a vital role in multiplication, growth, development and distribution of insects, and influence on their population dynamics (Dhaliwal and Arora, 2001 and Khan et al., 2003). Ye (2001) also reported that the fruiting duration and age of fruit affect on the fruit fly abundance.

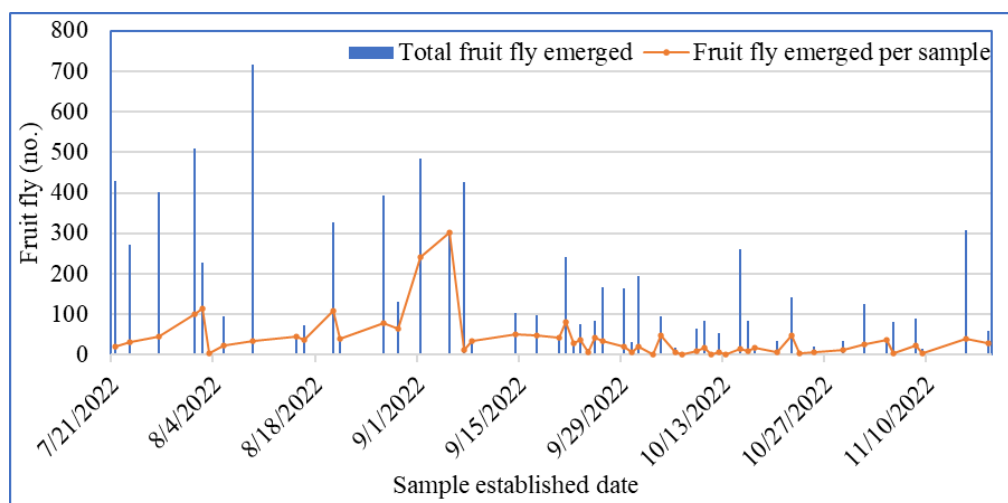
**Table 1.** Emergence of fruit flies from field infested flowers and fruits with respect to crops, growth stages of the host and damage intensity to host over two seasons, 2022

Attributes	No of fruit fly emerged	No of samples (n)	Fruit fly (No./sample)	$\chi^2$ goodness of fit
Cucurbit types				
Bitter gourd	611	46	13	$\chi^2$ (p<0.01)
Cucumber	681	30	23	
Sponge gourd	3016	59	51	
Pumpkin/Squash	3491	138	25	
Damage intensity to host				
Puncture noticed	1120	68	16	$\chi^2$ (p≤0.05)
Partly damaged	2770	82	34	
Severely damaged	3909	123	32	

Attributes	No of fruit fly emerged	No of samples (n)	Fruit fly (No./sample)	$\chi^2$ goodness of fit
Growth stages of host				
Male flower	155	59	3	$\chi^2$ (p≤0.01)
Female flower/post set fruit	3344	105	32	
Marketable fruit	4105	103	40	
Matured fruit	195	6	33	
Across two seasons				
Autumn-winter	954	55	17	$\chi^2$ (p≤0.05)
Summer-rainy	6845	218	31	
Grand Total	7799	273		

### Fruit fly species and their abundance in cucurbits

The grand average number of fruit flies emerged per sample was 29 irrespective to the cucurbit crops, fruit fly species and growth stages of the host (Fig. 4). The fruit fly number per sample was maximum (301) in marketable size sponge gourd during summer-rainy while the minimum (1) was in flower of pumpkin during autumn-winter. Narayanan & Batra (1960) reported that *Z. cucurbitae* remained active and bred throughout the year except for a short period of winter months and cause heavy fruit damage during July to August in various cucurbits.



**Fig. 4.** Total and average fruit fly emerged from the infested samples during summer-rainy and autumn-winter seasons, 2022.

A total of 7799 adult fruit flies emerged from the 273 cucurbit samples (Table 2). Four fruit fly species; *Z. cucurbitae*, *Z. tau*, *Z. scutellaris* and *Z. diversus* were observed and *Z. tau* was the most abundant species that accounted for 82.2% of the total emerged adults followed by *Z. cucurbitae* accounting 15.6% of total emerged adults. Of the total adult flies, 51.3% were females, slightly higher than male (48.7%). The chi-square test showed the significant differences ( $\chi^2$ ,  $p \leq 0.02$ ) between male and female of fruit flies irrespective of species while the trend was not consistent in all

species (Table 2). The sex ratio (male: female) varied from host to host. It was found that the sex ratio of all adults was 0.95 while it varied (0.64 to 5.50) with species to species. The varied sex ratio has also been reported between hosts (Laskar, 2013) and between species (Deguine et al., 2012).

**Table 2.** Number of fruit flies emerged from 273 samples of field infested cucurbit flowers and fruits collected during summer-rainy and autumn-winter season, 2022

Species	Female	Male	Sex ratio (Male:Female)	Total
<i>Z. cucurbitae</i>	577 (47.4)	640 (52.6)	1.11	1217 (15.6)
<i>Z. tau</i>	3337 (52.1)	3073 (47.9)	0.92	6410 (82.2)
<i>Z. scutellaris</i>	81 (60.9)	52 (39.1)	0.64	133 (1.7)
<i>Z. diversus</i>	6 (15.4)	33 (84.6)	5.50	39 (0.5)
<b>Total</b>	<b>4001 (51.3)</b>	<b>3798 (48.7)</b>	<b>0.95</b>	<b>7799 (100)</b>

Value in the parenthesis indicates the percent of total in respective row (male and female) and column (total).

Of 273 samples, 70.3% samples had only one species while 28% samples had two species and remaining 1.7% samples had three species emergences from infested flower/fruit rearing (Fig. 3). Budiyaniti et al. (2019) also found more than one fruit fly species in bitter gourd.

**Table 3.** Number of fruit fly species emerged from infested fruit/flower of cucurbits during summer-rainy and autumn-winter season, 2022

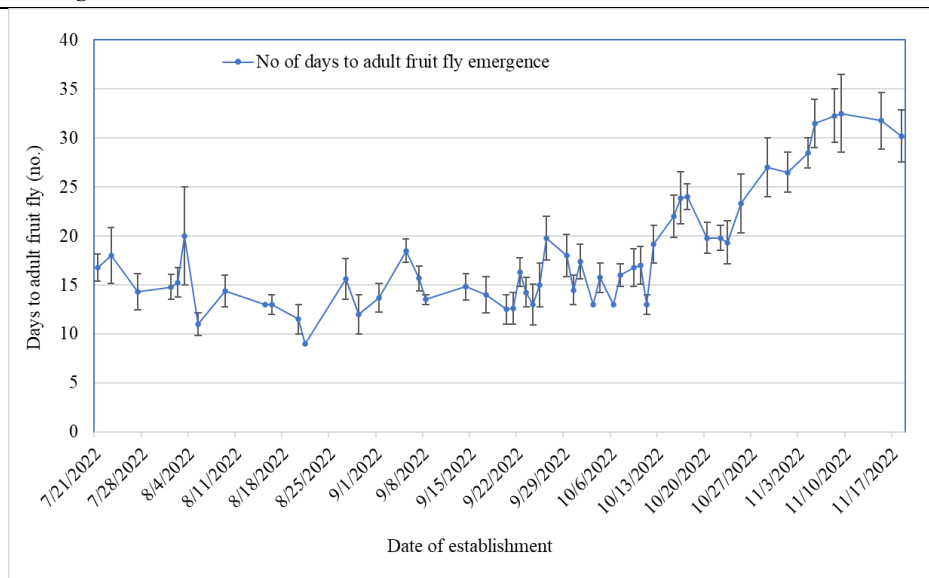
Cucurbit crop	No of fruit fly species emerged from cucurbit sample			Total Sample
	1	2	3	
Bitter gourd	36	10	0	46
Cucumber	27	3	0	30
Sponge gourd	46	12	1	59
Zucchini/pumpkin	83	51	4	138
<b>Grand Total</b>	<b>192 (70.3%)</b>	<b>76 (28%)</b>	<b>5 (1.7%)</b>	<b>273 (100%)</b>

### Fruit fly species dominance in cucurbits and locations

Flowers/fruits samples infested with different species of fruit fly with respect to crops, host growth stages and locations are presented in Table 4. Cucurbit crops were largely infested with *Z. tau*. except bitter gourd. *Z. tau* and *Z. cucurbitae* emerged from infested cucurbit fruits while *Z. scutellaris* and *Z. diversus* emerged from flowers and post-set fruit of zucchini/pumpkin and sponge gourd. *Z. tau* and *Z. cucurbitae* were common in all locations. *Z. cucurbitae* was dominant in Chitwan while *Z. tau* was dominant in other locations. Nair et al. (2017) also reported *Z. cucurbitae* and *Z. tau* as major fruit fly species infesting all of cucurbit fruits and flowers whereas *Z. diversus* infested flowers only including male flower of pumpkin (Tsuruta and White, 1997). The preference of fruit fly species had been reported in different hosts. *Z. cucurbitae* preferred bitter gourd for oviposition, immature feeding and adult emergences as compared to cucumber, pumpkin and muskmelon (Shahzadi et al., 2019). *Z. tau* preferred pumpkin as compared to cucumber and bitter gourd in choice test but the highest number of adult emergences was observed from cucumber in no-choice test (Karnjanaugkool and Julsirikul, 2021).

**Table 4.** Percentage of flower/fruit samples infested by fruit fly species in different crops, host growth stages and locations during summer-rainy and autumn-winter season, 2022

Cucurbit crops	Percentage of samples (n=273) infested by				More than one sp. (%)
	<i>Z. tau</i>	<i>Z. cucurbitae</i>	<i>Z. scutellaris</i>	<i>Z. diversus</i>	
Bitter gourd	43.5	78.2	0	0	21.7
Cucumber	70.0	40.0	0	0	10
Sponge gourd	81.4	39.0	3.4	0	22.0
Zucchini/pumpkin	44.2	33.3	47.1	18.1	39.9
<b>Growth stages of host</b>					
Male flower	3.1	15.6	76.6	37.5	32.8
Female flower/post-set fruit	68.0	56.0	18.0	1.0	43.0
Marketable fruit	71.8	48.5	0	0	20.4
Matured fruit	100	16.7	0	0	16.7
Grand average	54.9	42.9	24.5	9.2	29.7
<b>Sampling location</b>					
Sampling location	Percentage of total emerged fruit fly infested by				Total (No.)
	<i>Z. tau</i>	<i>Z. cucurbitae</i>	<i>Z. scutellaris</i>	<i>Z. diversus</i>	
Chitwan	6.8	93.2	0.0	0.0	367
Palpa	87.4	12.6	0.0	0.0	358
Rupandehi	90.7	9.3	0.0	0.0	1209
Sindhuli	99.5	0.5	0.0	0.0	617
Kathmandu Valley	83.3	13.3	2.6	0.8	5150
Mahottari	72.4	27.6	0.0	0.0	98
Grand average	82.2	15.6	1.7	0.5	7799

**Fig. 5.** Number of days required to emerge adult flies from fruit fly infested flowers/fruits during summer-rainy to autumn-winter season, 2022.



### Duration of adult fruit fly emergence

The number of days to emerge adult fruit flies from infested flowers/fruits samples establishment widely ranged from 7 to 43 (mode=14). This development period varied with seasons. It was only 14.6 days during summer-rainy season while it was longer, i.e. 22.2 days during autumn-winter (Fig. 5). The total development period from egg to newly emerged *Z. tau* adult was varied from 21 to 34 days (Liu & Lin, 2000).

### CONCLUSIONS

In 273 single flower/fruit rearing experiments, four fruit fly species: *Z. cucurbitae* (Coq.), *Z. tau* (Walker), *Z. scutellaris* (Bezzi) and *Z. diversus* (Coq.) were found as damage causing species in major cucurbits. Two fruit fly species, *Z. tau* and *Z. cucurbitae* were the dominant species in cucurbit fruits damage while *Z. scutellaris* and *Z. diversus* were dominant in cucurbit flowers damage including post-set fruits with flowers. More than 70% damages were caused by single species while about 30% were damaged by more than one species of fruit fly. Preference of fruit fly species differed with respect to location, crop and growth stages of host. Hence, management strategies should be developed targeting the dominant fruit fly species along with the crop, growth stages of host and location.

### ACKNOWLEDGEMENTS

International Foundation for Science, Sweden (IFS grant no. 1-3-C6185-1) is acknowledged for the financial support to this study. Prasamsha Pandey, Sabita Basnet and Pawan Dhakal are also acknowledged for their contribution in sample collection and laboratory works.

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