SAARC J. Agric., 22(1): 85-96 (2024) *DOI: https://doi.org/10.3329/sja.v22i1.69840*

LONGEVITY AND MORPHOMETRICS OF CHINESE CITRUS FLY, *BACTROCERA MINAX* (ENDERLIN) (TEPHRITIDAE: DIPTERA)

S. Sharma[1,2*], S. Tiwari[2], D. Adhikari[2,3] and B. Gautam[2]

[1] Plant Village Nepal
[2]Department of Entomology, Agriculture and Forestry University, Chitwan, Nepal
[3]Plant Quarantine and Pesticide Management Centre, Nepal

ABSTRACT

Chinese citrus fly, *Bactrocera minax*, (Enderlin) (Tephritidae: Diptera) is univoltine pest causing severe damage in citrus fruits. Longivity in different carbohydrate diets and morphometrics of adult Chinese citrus fly (CCF) was studied in uncontrolled laboratory room in Agriculture and Forestry University, Chitwan, Nepal. The result showed that the emergence of CCF was the highest (88%) for the pupa weighing more than 0.076 g with male and female emergence ratio of 1:2.1 followed by pupal weight of 0.049-0.076 g with emergence of 82.1%. The maximum population emerged at the last week of March, 2021. The adult emergence was recorded 66-90% at the additional moisture content of 15-20% in oven-dried soil with no adult emergence in completely oven dried soil. The adult longivity was 17.90 ± 1.80 days for female adult in 10% sucrose solution and was 15.09 ± 1.49 days for male adult in 10% jaggery solution. Length of Chinese citrus fly pupa was 7.46 ± 0.30 mm while breadth was 0.97 ± 0.004 mm with average weight of 0.078 ± 0.002 g. Similarly, length of female was 16.09 ± 0.27 mm including ovipositor (4.28 ± 0.41 mm) which was 43.8% higher than male fly (11.39 ± 0.16 mm). Wing span of male was 2.50 ± 0.006 cm and of female was 2.72 ± 0.10 cm where female had 8.8% wider wing span compared to male. The study findings are useful in comparing characters of *B. minax* with other fruit fly species to develop the specific management practices of CCF.

Keywords: CCF; Emergence; Longevity; Morphometrics

INTRODUCTION

Among fruits, citrus covers 23.6% of total fruit cultivated area of Nepal with the productivity of 9.47 tons per hectare (t/ha) (MoALD, 2022). Citrus occupies third position in total fruit production and has 3% share out of total fruit export by volume

* Corresponding author: sushmitasharma533@gmail.com

Received:12.11.2023 *Accepted: 19.04.2024*

from Nepal (Dahal et al. 2020). Citrus cultivation is highly profitable and advantageous in comparison to other crops in hilly regions of Nepal (MoAC, 2011). Among citrus, sweet orange is commonly cultivated in 54 districts of Nepal and the leading districts are Sindhuli (15.04 t/ha) and Ramechhap (13.65 t/ha) (MoALD, 2022).

Chinese citrus fly, *Bactrocera minax* (Enderlin) (Tephritidae: Diptera) has been considered as a major pest of citrus in Asia. Sweet orange is the most preferred host crop (Xia*et al.* 2018) and recently it has been reported that the pest infests mandarin orange along with lemons (Adhikari et al., 2022). This is a univoltine species and adult Chinese citrus fly has the ability of long-range flight (CABI, 2019) that was supposed to migrate from China to the mid-hills of Nepal through India (Sharma and Dahal, 2020). It is the most devastating oligophagous and univoltine insect of sweet orange in eastern hill of Nepal (Acharya and Adhikari, 2019; Adhikari et al., 2020). It is one of the largest dipteran citrus fruit fly species and morphologically similar to the Japanese fruit fly (*B. tsuneonis* Miyaka) (Drew and Romig, 2013). Infestation was seen more frequently in the orchards established above 1,100 meter above sea level and maximum damage was observed in Ramechhap and Sindhuli districts of Nepal (Chauhan et al., 2020). Adhikari et al. (2022) reported that the infestation has been seen extended to the 20 mid-hill districts including both eastern and western part of Nepal. Majority of farmers are confused between the Chinese citrus fly (CCF) (*B. minax*) and cucurbit fruit fly, *Bactrocera cucurbitae* (Coquillett, 1849) or oriental fruit fly, *Bactrocera dorsalis* (Hendel, 1912) and use the management strategies which are effective for the cucurbit fruit fly (Gautam et al., 2020). According to Adhikari et al. (2022), *B. minax* is the most problematic in citrus production and farmers are facing difficulties in managing the pest in the field.

The body length of female fly (14.29 mm) is more than male fly (12.52 mm) (Adhikari et al., (2022). Diatery nutrition plays important role in development and reproduction of adult flies. Lee et al. (2008) reported that high diatery carbohydrate to protein ratio increases the longevity of adult fly. Low protein and high carbohydrate diet intake by adult fly maximizes the life span of fly but feed with only protein diet or carbohydrate diet helps in optimal longevity of fly (Bruce et al., 2013). The life span of adult fly was found increased when adult was reared in higher altitude.

It is essential to have detailed information on the morphological characters and biology of the pest to identify the pest and this information will be helpful in determining the specific management strategy for controlling the pest (Adhikariet al., 2022). The result from the study will be beneficial in longevity and emergence study of CCF in lab condition with detailed understanding on the morphological parameters of adult CCFfor differentiating *B. minax* with other species.

MATERIALS AND METHODOS

Determination of adult emergence based on wight of pupa

The pupae were collected from Gulanjor, Sindhuli (27.2800° N, 86.0707° E) and were brought into the Entomology Laboratory of Agriculture and Forestry University in Chitwan. The pupae were kept in 2 plastic containers (200 ml capacity, 10 cm diameter and 20 cm height) with soil (150 g each) while collecting from Gulanjor, Sindhuli. Hundred randomly selected pupae from total collected pupae (250 pupae) were measured individually by using three-digit electronic weighing balance (Phoenix company). The measured pupae were categorized into three groups based on the pupal weight. The weight category was <0.049 g (19 pupa out of 100), 0.049g - 0.076 g (56 pupa out of 100) and >0.076 g (25 pupa out of 100). Pupae based on the weight were kept in 6 plastic containers (2 for each category of group) having the size of 40 ml capacity (7 cm diameter, 10 cm height with 25 g soil in each container). The plastic containers were covered with fine mesh net which was cut into the sizes just to cover the upper diameter of the container to ensure good air flow. Adult emergence percentage was observed from three categories (mentioned above) based on weight of pupa. Adult emergence date was recorded from each group of pupae.

Emergence of adult based on moisture treatment in soil

Effect of moisture was determined in late stage of pupa. Pupae were collected from 14th February to 20th February, 2020 from Gulanjor Rural Municipality-5. Total of 100 pupae were kept in the soil (soil type was clayey loam, collected from field of Gulanjor Rural Municipality, Sindhuli) which was kept in plastic container (40 ml capacity, 7 cm diameter and 10 cm height). Various moisture levels were maintained in oven dried soil (initial weight = 67.9 g after oven dry = 61.2 g) and kept in fluctuating room temperature. Five moisture levels were: 0% (water 0g, soil 61.2g), 5% (water 3.06 g, soil 61.2g), 10% (water 6.12g, soil 61.2g), 15% (water 9.19 g, soil 61.2g) and 20% (water 12.25 g soil 61.2g). Watering was done at 7-day interval. Five treatments were maintained in Completely Randomized Design (CRD) design. Each treatment was replicated 5 times with 4 pupae each and each moisture level contained 20 pupae. Adult emergence (male and female) from different moisture level was recorded to determine the percentage of adult emergence in various moisture level.

Longevity of adult Chinese citrus fly

Newly emerged adults were observed and separated on the basis of sex. Male and female CCF were separated and kept in plastic containers (50 mm length and 20 mm diameter). The lid of the plastic container had 10 holes to ensure good air flow. Five different carbohydrate-based food baits were used as treatment such as control (only water), sugar solution, sucrose solution, honey water solution and jaggary water solution. For feeding Chinese citrus fly, 10% sugar solution, 10% sucrose solution, 10% honey water solution and 10% jaggary solution were used using soaked cotton roll. In each treatment, there were 25 plastic containers (Hou et al., 2020). Each

plastic container contained one adult CCF. Chinese citrus fly was reared until its death to determine the longevity. The experiment was established in CRD design in a laboratory condition. The soaked cotton was changed in every two-day interval in each container. Date of emergence and date of death of both male and female adult was noted to determine the longevity of fruit fly. Lethal Time 50 (LT50) was calculated in terms of days where mortality of 13 adult Chinese citrus fly was considered as LT50.

Morphometric measurement of pupa and adult Chinese citrus fly

Different body parts of male and female Chinese citrus fly were measured with the help of vernier calipers (0-150 cm) and ocular microscope (Unilab India, IS: 4381:1357 microscope with 1 div = 0.1 mm in ocular micrometer and 1 div = 0.01 mm in stage micrometer) with 10X magnification. Overall body length, head, thorax, legs, abdomen and ovipositor in female was measured using digital vernier calipers while antennae and halter were measured by using ocular microscope. Altogether 50 adult flies (25 males and 25 females) were used for measuring morphological parts and the measurement was expressed in mm. Length and breadth of 100 pupae were measured in mm using vernier calipers and weight was taken using 3-digit electronic weighing balance (Phoenix company) and expressed in gm.

Calibration of ocular microscope was done and index was calculated by using the following formula.

Index = unit of stage/ unit of ocular ×10

Measurement in ocular microscope was noted and measured in micrometer and converted to millimeter by using conversion ration; 1 micrometer = 1/1000 millimeter

Wing span of male and female adult was measured by using vernier calipers and expressed in cm.

Statistical analysis

Data obtained from field and laboratory experiments were analyzed using Excel 2016, R studio version 20 (t-test, ANOVA). One way ANOVA was performed to check whether the data are statistically significant from one another. Mean comparison was done using Least Significant Difference (LSD) test at 5% level of significance. Percentage and frequency calculation was done to indicate percentage and numerical value of data. Percentage analysis and line graph was prepared using Microsoft Excel 2016.

RESULTS AND DISCUSSION

Adult emergence based on pupal weight in laboratory condition

Table 1 shows the emergence of pupae based on their weight. The highest emergence with lowest mortality of Chinese citrus fly pupa was recorded from > 0.076 g pupal

weight followed by 0.049 - 0.076 g. The least emergence of CCF adult was seen in < 0.049 g weight CCF pupa with male to female ratio of 1:1.2. Among the total emergence, the highest percentage of female adult (68.18%) was emerged from > 0.076 g pupal group while the highest male adult emergence (45.65%) was found from 0.046 - 0.076 g group. The mortality percentage of pupa was higher in the pupal weight of > 0.049 g. The emergence of male was less compared to female in all the pupal groups. The percentage emergence of adult from pupa was found directly related to the weight of pupa. Percentage of emergence of female was higher than male which supports the result of Thapaliya et al. (2020).

Table 1. Emergence of adult Chinese fruit fly on the basis of weight of pupa in fluctuating room condition

Weight (g) of pupa	No. of pupa	No. of adult fly emerged (%)	Mortality %	Male (%)	Female (%)	Male to female ratio
<0.049	19	11 (57.8)	42.2	5 (45.45)	6 (54.54)	1:1.2
0.049-0.076	56	46 (82.1)	17.9	21(45.65)	25 (54.34)	1:1.2
>0.076	25	22 (88)	12	7 (31.81)	15 (68.18)	1:2.1

Note: Value in parenthesis is percentage

Figure 1 shows the emergence of pupa in laboratory condition at room temperature. The highest emergence of male adult (11) was recorded on the 29 March 2021 while highest female adult emergence (14) was recorded on 30 March 2021. The figure shows that the adult CCF emergence trend was from 19 March, 2021 to 7 April 2021 while the emergence was decreasing from 31 March with zero emergence on 7 April. The number of female adult emergence was greater than the male which was 24% higher than male emergence. This result differs from Chauhan et al. (2019) who reported that the highest emergence in lab condition was recorded at the second week of March (i.e.17^{th}-18^{th} March) at an elevation of 1400-1474 meter above soil level.

Effects of moisture treatment on rearing media on adult emergence

Moisture treatment in soil significantly affected the emergence of adult Chinese citrus fly (P = 0.002) as shown in Figure 2. The CCF adult emergence was higher at the water content of 20% followed by 15%. There was significant decrease in emergence of adult fruit fly at 5% and 20% water content (P = 0.04). Adult emergence was zero when the water content in soil was 0% (Figure 2).

From the study, it was found that the emergence percentage was higher up to 20% of moisture content in oven dried soil. The least percentage of emergence (0%) was from complete oven dried soil which was not treated with additional water. On the contrary to this result, Liet al. (2019) reported higher percentage of emergence at 15% additional water content and emergence decreased at 20% of additional water content.

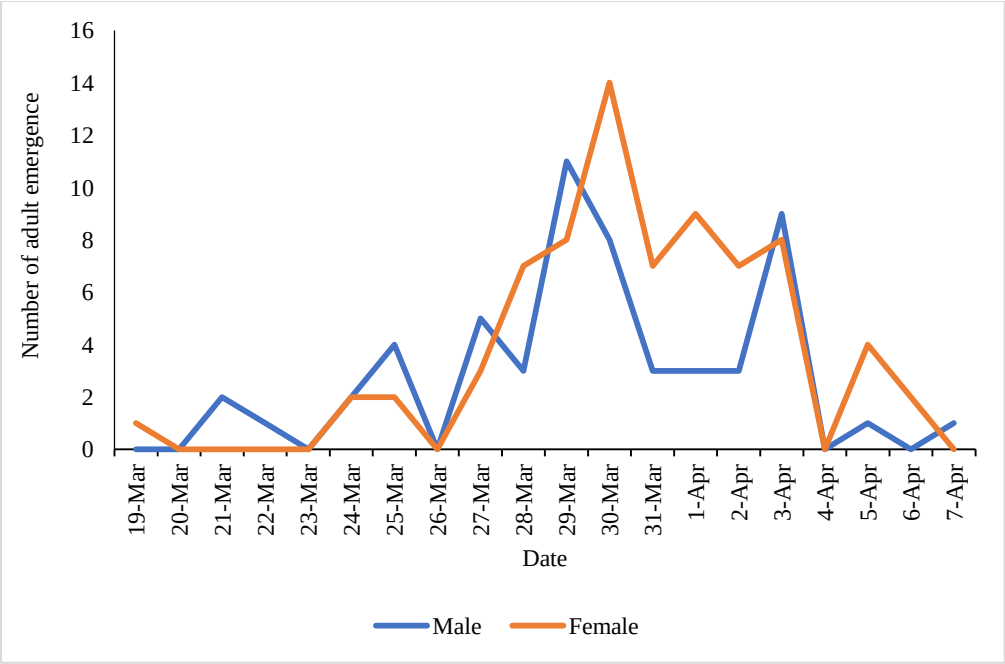

Figure 1. Emergence of adult Chinese citrus fly in the room condition at different dates in 2021

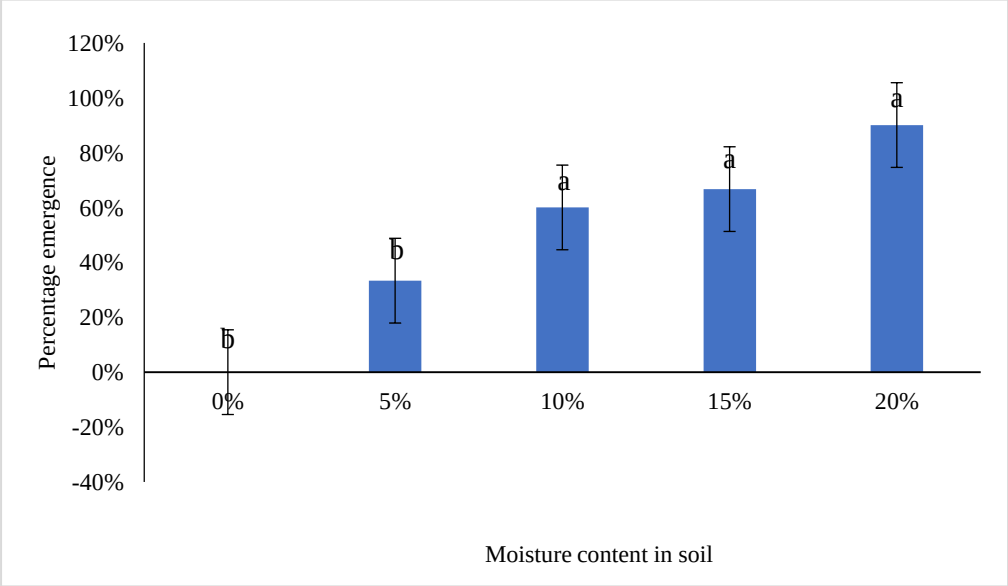

Figure 2. Emergence of adult Chinese citrus fly in different moisture content of soil at room condition

Longevity study of adult Chinese citrus fly

Diet significantly influenced the longevity of male adult ($P < 0.001$) and female ($P = 0.000$) adult Chinese citrus fly (Table 2). In male, the longest life expectancy had been observed when fed with jaggery solution (15.09 ± 1.49 days) which was not significantly different to the sucrose solution (14.72 ± 2.08 days, P=0.40) and sugar solution (14.18 ± 1.96 days, P=0.79) and all of which was significantly different with honey (8.36 ± 1.13 days, P=0.01) and water (6.09 ± 0.7 days, P=0.000).

In female, the longest life expectancy was observed when fed with sucrose solution (17.9 ± 1.8 days) which was significantly similar to the jaggary solution (15.3 ± 1.5 days, P = 0.12). The result was found statistically different when fed with sugar solution (13.2 ± 1.5 days, P = 0.02), honey solution (12.6 ± 1.05 days, P=0.003) and water (9.1 ± 0.8 days, P = 0.000) (Table 2).

The longevity of male and female was tested in different carbohydrate diets in which the longest life expectancy of male was found when fed with 10% jaggery solution while in female the longest life expectancy was seen when fed with 10% sucrose solution. The life expectancy of female from the study was (17.9 ± 1.8 days) which was found slightly different from the results of Wang et al. (2018) where life expectancy in sucrose diet was 19 days. However, life expectancy in water was similar with Wang et al. (2018). There was 100% mortality after 25 days in all the carbohydrate diets which supports the result of Wang et al. (2018). According to the study of 219 inbred lines of *Drosophila*, Arya et al. (2010) reported that about 70% of unmated female had longer life span as compared to unmated males. Similarly, Regan et al. (2016) suggested that difference in the intestinal stem cell activity can be the responsible factor in higher longevity of female fly as compared to male fly.

Table 2. Life expectancy of adult fruit fly in different artificial diet in Lab condition

Diet	Life expectancy (days)	
	Male	Female
Sugar	14.18 ± 1.96^{a}	13.20 ± 1.5^{b}
Honey	8.36 ± 1.13^{b}	12.60 ± 1.05^{b}
Sucrose	14.72 ± 2.08^{a}	17.90 ± 1.8^{a}
Jaggery	15.09 ± 1.49^{a}	15.30 ± 1.5^{ab}
Water	6.09 ± 0.7^{b}	9.10 ± 0.8^{c}
CV%	12.59	9.76
SEM ±	0.29	0.26
LSD	4.045	2.62
F-value	4.91*** (p = 0.000)	6.95*** (p = 0.002)

Note: *** significance at 0.1% level, LSD =Least Significance Difference, CV = Coefficient of variation, SEM = Standard Error of Mean,

Mortality of adult was observed in different carbohydrate diets. The mortality rate was found the highest when fed with water followed by honey (Table 3). Mortality was found 100% in water and honey at 20 days, jaggery, sugar and sucrose at 25 days. The shortest LT50 (Lethal Time 50) was found in the adults fed with water (5.5 days) while longest LT50 value was found in sucrose solution (12.6 days) which was followed by Jaggery (10.25 days), sugar (8.3 days) and honey (6.6 days). Mortality was extended up to 25 days in sucrose, sugar and Jaggery (Table 3).

Table 3. Cumulative adult mortality in different artificial diet in lab condition

Diet	5-day	10-day	20-day	25-day	LT50 (Days)
Water	28%	84%	100%	100%	5.5
Honey	17.8%	57.1%	100%	100%	6.6
Jaggery	0%	26.07%	91.3%	100%	10.25
Sugar	0%	36.8%	76.9%	100%	8.3
Sucrose	0%	35%	65%	100%	12.6

Morphometric measurement of pupa and adult of Chinese citrus fly

Table 4 shows the mean length, diameter and weight of pupa. Average length of Chinese citrus fly pupa was found 7.46 ± 0.3 mm while breadth was 0.97 ± 0.004 mm. The average weight of pupa was found 0.078 ± 0.002 g. The ratio of length and diameter of CCF pupa was found 7:1. As reported by Mir et al. (2014), the length of *B. cucurbitae* pupa was measured 5.72 ± 0.13 mm which was shorter than *B. minax.* The study slightly differs with Zhang (1989) which reported higher length (9-10 mm) and diameter of pupa (4 mm).

Table 4. Measurement of pupa of Chinese citrus fly

Parameters (n=100)	Mean ± SD
Length (mm)	7.460 ± 0.300
Breadth (mm)	0.970 ± 0.004
Weight (g)	0.078 ± 0.002

The overall length of female is higher than male which is about 43.8% (Table 5). Overall body breadth with wingspan of female is higher than male with overall wing span of male (2.5 ± 0.006 cm) and female (2.72 ± 0.10 cm). The length and breadth of head and eye in male and female is similar. Length of foreleg in male and female is same while breadth of foreleg in female is slightly higher than male. Length of meso-thoracic leg is higher in female fruit fly while breadth is found higher in male. Similarly, length of hind leg is higher in female however breadth is similar in both

sexes. Length of wing is nearly similar in both male and female while breadth of wing is higher in female as compared to male. The length and width of antennae and halter is found similar in both sexes (Table 5). In female length breadth ration of ovipositor was 6:1.

The overall length of male was found 11.39 ± 0.16 mm while female was 16.09 ± 0.27 mm with length of ovipositor 4.28 ± 0.41 mm. This result supports the findings of Dorji et al. (2006) who suggested that the length of adult fly was about 10-13 mm without including ovipositor. Besides, Adhikari et al. (2022) reported that the mean body length and average wing length of female fly was higher as compared to male fly. Comparing the overall length of *B. minax* and *Z. cucurbitae*, the length of *Z. curcurbitae* was shorter for male (8.74 ± 0.32 mm) and for female (9.94 ± 0.20 mm) as reported by Miret al (2014). In comparison to the wing length of *B. minax* and *B. dorsalis*, the wing length of adult was shorter in *B. dorsalis* (6 mm) as reported by Drew et al (2008).

The findings revealed that there was no significant difference in wing span according to sex of Chinese citrus fly. However, female had 8.8% wider wing span as compared to male (Table 5). Wing span was found 2.5 cm which differs from Dorji et al. (2006) who reported 10 mm wing span of *B. minax*. This result is similar with the report of Regmi et al. (2023) which showed 16-24 mm range of wing span in adult fly and wing span of female (23.14 mm) was found higher as compared to male (20.71 mm). Comparing the wing span with *B. cucurbitae*, the wing span was shorter in *B. cucurbitae* for male (10.97 ± 0.43 mm) and for female (13.02 ± 1.28 mm) as reported by Akter and Sohel, (2020).

Table 5. Morphometrics of adult Chinese citrus fly (n = 25)

Body parts	Length (mm) Mean ± SD		Breadth (mm) Mean ± SD	
	Male	Female	Male	Female
Overall body	11.39 ± 0.16	16.09 ± 0.27	-	-
Overall body breadth with wingspan	-	-	27.75 ± 0.08	30.18 ± 0.1
Head	2.70 ± 0.16	2.42 ± 0.14	2.83 ± 0.10	2.50 ± 0.16
Eye	1.78 ± 0.11	1.55 ± 0.13	0.96 ± 0.07	0.87 ± 0.08
Fore leg	6.82 ± 0.24	6.81 ± 0.27	0.38 ± 0.03	0.40 ± 0.02
Mesothoracic leg	9.63 ± 0.25	10.13 ± 0.33	0.42 ± 0.01	0.39 ± 0.02
Hind leg	8.61 ± 0.31	9.06 ± 0.37	0.39 ± 0.03	0.39 ± 0.02
Wing	8.44 ± 0.17	8.83 ± 0.17	2.78 ± 0.08	3.92 ± 0.17
Thorax	4.59 ± 0.09	4.34 ± 0.11	3.05 ± 0.12	2.94 ± 0.12
Abdomen	5.87 ± 0.12	5.56 ± 0.18	2.39 ± 0.06	3.52 ± 0.13

Body parts	Length (mm) Mean ± SD		Breadth (mm) Mean ± SD	
	Male	Female	Male	Female
Antennae	1.18 ± 0.02	1.22 ± 0.02	0.52 ± 0.02	0.58 ± 0.02
Arista	1.31 ± 0.02	1.37 ± 0.03	-	-
Halter	0.84 ± 0.02	0.77 ± 0.03	0.42 ± 0.02	0.47 ± 0.03
Ovipositor	-	4.28 ± 0.41	-	0.70 ± 0.11

CONCLUSION

Chinese citrus fly is a major invasive pest in citrus crops. Pest starts emerging in the field after temperature starts increasing and emergence is seen from the month of March. The weight of pupa also affects the emergence percentage where higher weight resulted higher emergence. Moisture content in the soil has direct influence in the emergence whereby there was no emergence at dried soil. Carbohydrate diets are also determining factors for estimating longevity of the adult fly. Life expectancy of female Chinese citrus fly is higher in sucrose solution while male longevity was higher in jaggery solution. The length, diameter and wing span of female adult Chinese citrus fly is greater compared to adult male fly. The present study can be informative in differentiating different species of fruit flies affecting the citrus from *B. minax* and this information can be utilized for the management of Chinese fruit fly in citrus growing orchards.

ACKNOWLEDGEMENT

We would like to acknowledge the Ministry of Agriculture and Livestock Development (MOALD) of Bagmati Province, Nepal for aiding with the fund for the research activities.

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