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ADULT EMERGENCE AND MORPHOMETRICS OF CHINESE CITRUS FLY, *Bactrocera minax* (Enderlein) (DIPTERA: TEPHRITIDAE) IN NEPAL

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Abstract

Chinese citrus fly, *Bactrocera minax* is a destructive and univoltine pest of citrus fruits. Geographical altitudinal gradients as well as prevailing climate affect the biology and ecology of insect. Hence, this study aimed to ascertain the effect of altitude on the adult emergence and morphological variations in various ecological settings of Ramechhap district of Nepal from February to June 2021 in citrus orchard. Six altitude ranges were selected in 50 m distance from 1200 to 1500 m above sea level (masl), ranging from 1201-1250 masl, 1251-1300 masl, 1301-1350 masl, 1351-1400 masl, 1401-1450 masl and 1451-1500 masl. The peak adult emergence periods were the 2nd, 3rd, 4th week of April in 1201-1250 masl, 1251-1300 masl, 1301-1350 masl, respectively, followed by 1st, 2nd and 3rd week of May in 1351-1400 masl, 1401-1450 masl, 1451-1500 masl, respectively. Morphometrics of Chinese citrus fly such as weight, length and width of pupa and adult were almost similar to the species collected in various altitudes. The average body length of male Chinese citrus fly adult was 11.58± 0.112 mm while female was 15.57± 0.076 mm. The average wingspan of male was 20.71± 0.285 mm while that of female was 23.14± 0.156 mm. The longevity of adult Chinese citrus fly species increased with increase in altitude. This information could be useful to design an appropriate management plan of Chinese citrus fly in various altitudinal gradients of Nepal.

Keywords: Adult emergence, Length, Longevity, Peak period, Sweet orange

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Introduction

Sweet orange, *Citrus sinensis* (L.) Osbeck is a high-value fruit having huge potential for area expansion in Nepal (Pun and Thakur, 2018). It ranks second among the citrus fruit species in terms of area and production after Mandarin in Nepal (Parajulee *et al.*, 2021). The geographic position and climate of Ramechhap district resembles with the mid-hills of Nepal that favors all citrus crops including sweet orange production (Acharya and Shrestha, 2021). Insect pest and diseases are the major production threat in citrus fruits in Nepal. Fruit flies (Diptera: Tephritidae) are among the most destructive agricultural pests that induce significant losses in production (Bhattacharya *et al.*, 2013). The distribution of fruit flies is cosmopolitan and distributed in tropical, subtropical and temperate regions (Agrawal and Sueyoshi, 2005). This pest can cause direct damage to fruits and vegetables by laying their eggs inside the fruits that lead to yield loss ranges from 90-100% depending on the various geographical, potential host and climatic conditions (Kumar *et al.*, 2011). Sixteen economically important fruit fly species are recorded in Nepal among them seven fruit fly species are reported from citrus orchards of Sindhuli (Adhikari *et al.*, 2019). Chinese citrus fly, *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) is an economic insect pests which attacks fruits of citrus species (Sharma *et al.*, 2015). In Nepal, this pest is distributed exclusively from eastern to central hilly areas of Nepal (Bhandari *et al.*, 2017). Chinese citrus fly was first reported in 2007 in Sweet orange at Paripatle, Dhankuta, district of Nepal (Adhikari *et al.*, 2020). This insect species are oligophagous, feeding exclusively on the fruits of citrus species and is univoltine unlike other tephritid flies, with adults emerging during April to May and overwintering as pupae (Xia *et al.*, 2019). The pest can cause up to 100% damage to the sweet orange fruit in severe condition (Huang *et al.*, 2007).

Fruit fly life stages are affected differently with respect to the altitude due and prevailing climatic conditions (Dominiak *et al.,* 2006). The assessment of adult emergence period of Chinese citrus fly is felt necessary for adoption of suitable management strategy in sweet orange orchards (Acharya and Shrestha, 2021). Assessment of adult emergence period and its behavior are the most fundamental requirement for successful pest management. It is equally important to ascertain pest identity and obtain knowledge on its habitat and behavior before applying any pest management strategies. Specifically, knowledge about the morphometrics of pupa and adult of the Chinese citrus fly can help sweet orange growers to manage the pest by making the identification easier and the implementation of control measures effective (Adhikari *et al.*, 2022). This study was carried out to know the adult emergence time at various elevations and document morphometrics of pupa and adult Chinese citrus fly in Ramechhap district.

Materials and methods

Research site

This study was conducted in Sunapati Rural Municipality and Ramechhap Municipality of Ramechhap district of Nepal. Study consisted of two major parts: study of adult emergence in various altitudes and morphometrics study of pupa and adult Chinese citrus fly. The experiment was conducted in randomized complete block design with six treatments (different altitudinal ranges) replicated four times.

Table 1. Location detail of adult emergence study in Ramechhap, 2021

Locations	Altitudes (masl)	Latitudes (°N)	Longitudes (°E)
Kartike	1201-1250	27.3312	86.0779
Babiyakharka	1251-1300	27.3338	86.0689
Dharapani	1301-1350	27.3335	86.0681
Bhangeri	1351-1400	27.3413	86.0614
Tallo Hileypani	1401-1450	27.3190	86.1074
Mathillo Hileypani	1451-1500	27.3185	86.1114

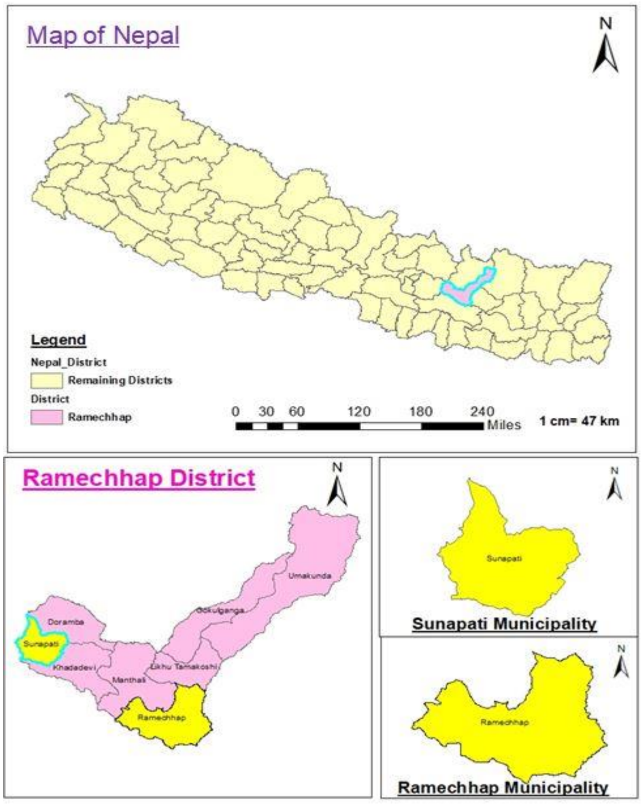

Figure 1. Map showing study site in Ramechhap district of Nepal

Assessment of adult emergence period

Pupae of Chinese citrus fly were collected from the sweet orange orchard of respective elevations on 10th of February, 2021. Five plastic containers (height 15.6 cm, length 9.4 cm and diameter 7.4 cm) were taken for each treatment. Each plastic container was filled with 40 g sandy loam soil from the respective orchard to its one-third portion. In each plastic container twenty Chinese citrus fly pupae were kept and then it was covered with sandy loam soil. In total, there were 80 similar size pupae for each treatment. Each plastic container was covered with 1.2 mm mesh size nylon net in order to prevent the escape of adult after its emergence. The soil was replaced with the respective orchards soil at an interval of one week in each container in order to assure similar field moisture inside the plastic container (size same as above).

The adult emergence of individual Chinese citrus fly from its pupae in the plastic container was recorded in its respective location. For this, the number of adult emergence was counted from among all the pupae of plastic container separately at its respective elevation. The date of adult emergence was recorded precisely. Then the period of adult emergence from eighty pupae each at its respective elevation was recorded which was further analyzed by using SPSS (v20) for the peak period of its adult emergence from pupae at their respective orchard.

Morphometrics of pupa and adult Chinese citrus fly

For assessment of morphometrics of pupa and adult of Chinese citrus fly, single pupa was kept in each plastic container and each container was filled with soil from respective orchard following the same procedure as for study of adult emergence period. Weight of pupae and adult were recorded using weighing balance (CocinaCo SF-400 C Electronic Compact Scale). Length and width of pupae were measured with the help of Vernier Caliper (Dasqua 150 mm measuring stainless) before keeping it inside the plastic container. The mortality of individual adult in each container was recorded daily. Body length and the expanded wingspan of both male and female adult Chinese citrus flies were measured with the help of measuring Ruler (Starrett C636ME 150 mm Steel Ruler). The sex ratio of adult in each replication was noted as per the formula:

$$\text{Sex Ratio} = \frac{\text{Total number of female emerged}}{\text{Total number of male emerged}} \text{ (Wei, 2010)}$$

Statistical analysis

Data was analyzed by RStudio (v2021). One-way ANOVA was used to check if the treatments were statistically different from one another. Square-root transformation was performed for data normalization. Mean comparison was done by using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Results and discussion

Adult emergence period

The highest frequencies (22) of adult Chinese citrus fly emergence were observed during second week of April at the altitude of 1201-1250 masl (Figure 2). In the altitude range of 1251-1300 masl, the maximum frequency (28) of adult emergence was observed during the third week of April. Maximum frequency (26) of adult emergence was observed during the last week of April in an altitude range of 1301-1350 masl. The maximum frequency (31) of adult emergence was observed during the first week of May in the altitude range of 1351-1400 masl. At an altitude of 1401-1450 masl, the highest frequency (30) of adult Chinese citrus fly emergence was observed in the second week of May. Similarly, altitude range of 1451-1500 masl, the maximum frequency (27) of adult emergence was observed during the third week of May. From the multiple line mean, it can be assumed that adult emergence of Chinese citrus flies have been shifted forward at weekly intervals with increase in altitude. Similar results were observed by Gautam *et al.* (2020) where in the early emergence of the Chinese citrus fly has been observed at lower altitude. In the higher altitude, longer pupal stage of Chinese citrus fly was observed by Wang and Luo (1995) which support the late adult emergence at higher altitude.

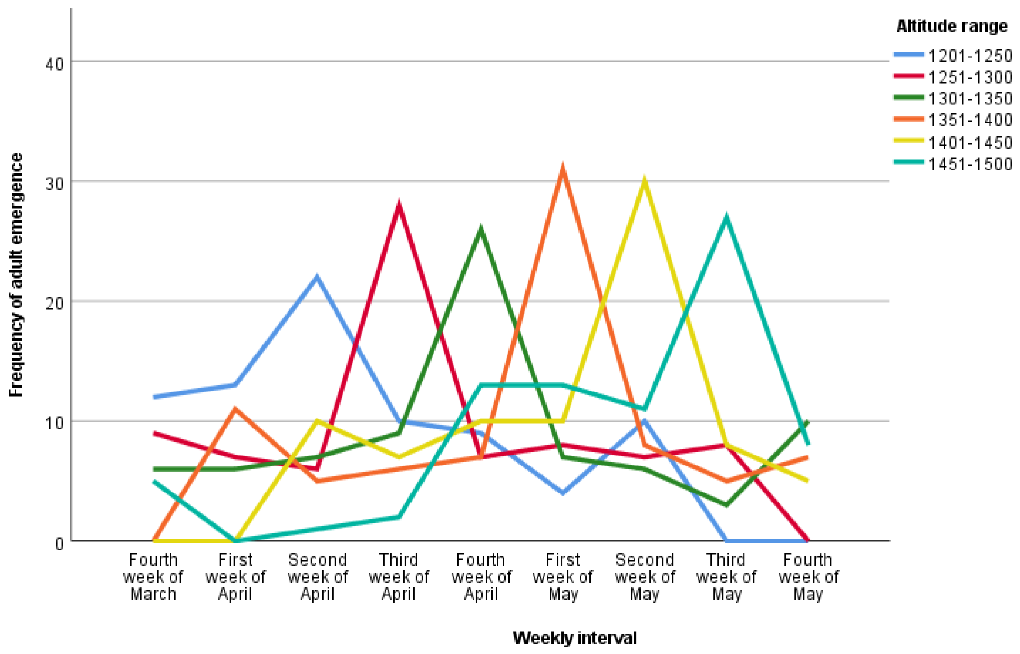

Figure 2. Frequency of adult emergence of Chinese citrus fly in Ramechhap from 4th week of March to 4th week of May, 2021.

Adult longevity and Adult Sex Ratio

There was non-significant effect of altitude on the sex ratio of adult Chinese citrus fly (Table 2). However, it was observed that sex ratio of Chinese citrus fly was similar regardless of variation of differences in the altitude. Similar result was observed in adult of Queensland fruit fly where the interaction between latitude and sex was non-significant in the analysis of covariance (ANCOVA) (Dominiak *et al.*, 2006). Female population was greater than male population. There was a significant effect of altitudinal variation on the longevity of adult Chinese citrus fly. Adult longevity was similar in altitudes of 1201-1250 masl, 1251-1300 masl, 1301-1350 masl, 1351-1400 masl, and 1401-1450 masl. Similarly, it was observed that longevity of adult Chinese citrus flies was found to increase with increase in altitude, highest being at 1451-1500 masl followed by 1401-1450 masl, 1351-1400 masl, 1301-1350 masl, 1251-1300 masl and 1201-1250 masl, respectively. Similar finding was observed in Ceratitis fruit fly by Duyck *et al.* (2010). Such variations can be affected by temperature (Danjuma *et al.*, 2014).

Table 2. Effect of different altitudes on longevity and sex ratio characteristics of adult of Chinese citrus fly in Ramechhap, 2021.

Altitude (masl)	Characteristics of adult	
	Longevity (days)	Sex Ratio (Female: Male)
1201-1250	2.05[b]	2.54
1251-1300	2.35[b]	1.09
1301-1350	2.45[b]	2.23
1351- 1400	2.45[b]	1.92
1401- 1450	2.45[b]	2.54
1451- 1500	2.95[a]	1.29
LSD (0.05)	0.393**	NS
SE_m (±)	0.073	0.28
P value	<0.01	>0.05
C.V.%	10.79	74.84
Grand Mean	2.45	1.94

SE_m: Standard error of means; LSD: Least significant difference; C.V.: Coefficient of variation; NS= Non-significant, **= significant at 1% probability

Morphometrics of pupa and adult of Chinese citrus fly

Table 3 shows the effect of different altitude on morphometrics measurement such as weight, length and width of pupa and adult of Chinese citrus fly in Ramechhap. No significant effect was observed between different altitude and morphometrics of pupa and adult of Chinese citrus fly (Table 3). Since there was no significant difference between morphometrics of pupa and adult, it was observed that morphometrics measurement such as weight, length and width of pupa and adult of Chinese citrus fly was found to be similar regardless of variation of difference in the altitude. Thus site specific population differentiation of Chinese citrus fly is not strongly supported by this data. Similar result was observed by Gomez, *et al.* (2014) where wing size was reported to be similar among populations of *Anopheles albimanus* (Culicidae: Diptera) with no significant differences between eco-regions.

Table 3. Effect of different altitudes on morphometrics of pupa and adult of Chinese citrus fly in Ramechhap, 2021.

	Morphometrics of pupa (n=120)			Morphometrics of adult (n=120)		
Altitude (masl)	Weight (g)	Length (mm)	Width (mm)	Weight (g)	Length (mm)	Wing span (mm)
1201-1250	0.07	10.00	4.26	0.016	14.19	22.62
1251-1300	0.08	10.04	4.34	0.017	13.39	21.54
1301-1350	0.06	9.89	4.37	0.016	13.81	22.30
1351- 1400	0.07	9.84	4.15	0.018	14.03	21.74
1401- 1450	0.08	9.94	4.13	0.015	14.18	22.38
1451- 1500	0.07	9.75	4.07	0.016	14.07	22.30
LSD (0.05)	NS	NS	NS	NS	NS	NS
SE_m (±)	0.001	0.04	0.04	0.0004	0.18	0.17
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
C.V.%	9.05	1.85	1.69	12.18	6.89	16.51
Grand Mean	0.07	9.9	4.22	0.016	13.94	22.15

SE_m: Standard error of means; LSD: Least significant difference; C.V.: Coefficient of variation; NS= Non-significant

Figure 3. Pupa of Chinese citrus fly collected from sweet orange orchard in Ramechap district.

Figure 4. Adult of Chinese citrus fly (*Bactrocera minax*) (Left: Female, Right: Male).

Morphometrics variation among male and female

The length of male adult Chinese citrus fly was found in the range of 9 to 12.5 mm while that of female was 13.0 to 16.6 mm (Table 4). Similar findings were observed by Adhikari *et al.* (2022) where length of male adult Chinese citrus fly was reported 9.47 to 14.45 mm and that of female ranged from 11.6 to 16.10 mm. Likewise, the wingspan of male adult Chinese citrus fly was 16.0 to 24.4 mm while that of female was ranges from 18.0 to 26.0 mm. Adults body length and wingspan was longer in females than male adult Chinese citrus flies. The length of male was smaller than the female which was similar with the findings of Sharma and Tiwari (2020). The female-biased body size dimorphism is well explained by the studies on Caribbean fruit flies indicating that the female had a longer developmental period or faster growth rates than males (Sivinski and Calkins, 1990). Similarly, smaller body size of male is believed to provide a high level of easy flight movement while mating as observed by Sivinski and Dodson (1992). Based on this finding, it can be suggested that Chinese citrus flies with longer wingspan and length are generally female. Similar findings were also reported by Adhikari *et al.* (2022). Such information is helpful to sweet orange growers in distinguishing adult Chinese citrus flies.

Table 4. Morphometrics of adult of Chinese citrus fly in Ramechhap, 2021 (n= 80).

Sex of adult	Length (mm)		Wingspan (mm)	
	Mean ± SE	Range	Mean ± SE	Range
Male (with expanded wings)	11.58 ± 0.112	9.00 – 12.5	20.71 ± 0.285	16.00 – 24.40
Female (with expanded wings)	15.57 ± 0.076	13.00 – 16.6	23.14 ± 0.156	18.00 – 26.00

Conclusions

In lower altitudes, the adult flies emerge earlier than in higher altitudes. Adult emergence period was shifted one week later with every 50 m rise in altitude. These findings clearly show that the life cycle of Chinese citrus fly is related to the location which is crucial in determining the timing to adopt the management strategy. The longevity of adult Chinese citrus flies increased with increase in altitude. Thus these results suggested that citurs orchard should be established at an altitude lower than 1450 masl. Pupal length and adult's wingspan of male were smaller than female. This information can suggest for designing an appropriate management strategy of Chinese citrus fly in various altitudes.

Authorship contribution

AS, DA and BR have conceived and planned the experiment. BR and SS carried out the experiments. ST and HNL were involved in planning and supervising the work. BR performed the analysis. ST, DA, JP and SP worked on the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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