

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

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ABSTRACT

The study was performed at the citrus orchard of Chhahari Retreat in Budhanilkantha, Kathmandu, examined the morphological measurements of both pupa and adult of *Bactrocera minax*, with a study from March to July 2023. The average pupal length of the Chinese citrus fly determined 9.44 ± 0.10 mm, with a corresponding breadth of 4.33 ± 0.09 mm and weight of 0.051 ± 0.002 gm. Notably, the body length of adult female *B. minax* surpassed that of males, measuring at 13.9 ± 0.20 mm compared to 11.93 ± 0.33 mm for males, while females exhibited broader bodies (3.92 ± 0.06 mm) in contrast to males (3.18 ± 0.05 mm). Furthermore, adult females displayed a wider wingspan (22.21 ± 0.23 mm) relative to adult males (21.36 ± 0.30 mm), along with longer wings (9.22 ± 0.16 mm) compared to males (8.86 ± 0.16 mm). The ovipositor length was 4.62 ± 0.62 mm. The average weight of female adults calculated 0.0228 ± 0.001 gm, whereas male adults exhibited an average weight of 0.0141 ± 0.002 gm. This research emphasizes significant morphological differences between male and female flies. Furthermore, an attempt was made to manage this pest through the implementation of Area-wide Control Program (AWCP), involving the application of a lethal protein bait comprising 25% protein hydrolysate and 0.1% abamectin (Great fruit fly bait), through spot application at weekly intervals from 8th May to 24th July 2023. A notable decline in fruit loss was observed, decreasing to 1.24% from the previously recorded 27.68% during the 2023 harvest season. This substantial reduction in overall fruit loss, amounting to a noteworthy 26.44% decrease in comparison to 2022 ($p < 0.00$), was effectively attained.

Keywords: Area-Wise Control Program, *Bactrocera minax*, morphometric, protein bait

INTRODUCTION

Citriculture, the cultivation of citrus fruits, holds cultural significance in Nepal, particularly with fruits like Mandarin (*Citrus reticulata*) and Sweet Orange (*Citrus sinensis*) traditionally thriving in the mid hill region. The country indeed serves as a hub for citrus diversity, nurturing numerous species within its borders (Adhikari and GC, 2020). In Nepal, citrus was traditionally grown in the mid-hill's region, but nowadays, it is also grown commercially in the Terai region (Acharya and Adhikari, 2019). Citrus cultivation is done in 66 districts covering 49,306 ha of area (MoALD, 2023). Citrus crops attract many insects, with tropical regions hosting up to three times as many pests as highland and Mediterranean areas due to warmer temperatures and humidity (Hussain et al., 2022). The primary insect pests affecting citrus crops include the fruit flies, Asian citrus psyllid, citrus leaf miner, thrips, green stink bug, as well as fruit piercing moths and mites. These pests collectively pose significant challenges to citrus cultivation (FAO, 2021). Research and development organizations dedicated to citrus fruit cultivation must explore novel and environmentally friendly chemical and cultural solutions to effectively address these emerging challenges (Adhikari, 2023).

The Chinese citrus fly (*Bactrocera minax* (Enderlein)) is a highly destructive pest in south-central China, capable of causing complete fruit loss under severe conditions. It has also spread to nearby regions, affecting orchards in Bhutan, northwest India (Sikkim and West Bengal), and Nepal (Xia et al., 2018). This fly's range expanded from China to Nepal through Bhutan and India. Geographically, it is limited to China, Bhutan, India (Sikkim and West Bengal), and Nepal (CABI, 2020). Contrary to the majority of pest species, the males of this species of *Bactrocera* are not attracted to any known chemical lures. Both sexes can be observed using protein bait traps (either protein hydrolysate or protein autolysate), although they also catch a lot of unrelated insects. *B. minax* infestation levels are consistently high, which emphasizes the need for a deeper understanding of its biology and ecology in order to develop and apply more efficient control strategies (Dong et al., 2014). This research has been conducted to investigate the morphology and assessment of the implementation of management strategies for the Chinese citrus fly in citrus orchards in Kathmandu, Nepal.

MATERIALS AND METHODS

Research site: The study was conducted in a problematic citrus orchard in Kathmandu, chosen for its location and compatibility with the field owners, as well as cost and proximity considerations. The site was situated at Chhahari Retreat in Budanilkantha at 1485.17 meters above sea level (27.7863210° N, 85.3542847° E).

Rearing of pupae for adult emergence: A total of 64 maggots were collected on 10th November 2022 from infested sweet oranges. Six transparent cylindrical containers, each 10 cm tall with a diameter of 4 cm, made of smooth, clear plastic, allowing full visibility of the contents, were used. The containers were filled two-thirds with loamy soil, and maggots were placed inside the soil. To prevent the escape of adult flies while allowing for ventilation, the containers were covered with muslin cloth. After the adults emerged, they were used to measure significant morphometric parameters.

Measurement of morphological features of pupae and adult specimens: Morphological analysis of pupae and adult specimens aimed to understand visible attributes during these life stages. A manual Vernier Caliper was used to measure body length, breadth, wingspan, wing length, and female fly ovipositor length as shown in Figure 2. Body length was measured from head to abdomen tip, while breadth was assessed at the mesothorax. Wingspan was measured from one forewing tip to the other, and wing length from joint to tip. Ovipositor length was gauged from abdomen joint to tip. These measurements were conducted to capture various morphological features and dimensions of the studied specimens (Adhikari et al., 2022a). The weight of both pupae and adult specimens was documented using a digital weighing balance (SINKO Level CIE 320g*0.001g) and (OHAUS analytical balance) respectively for precise weight measurements.

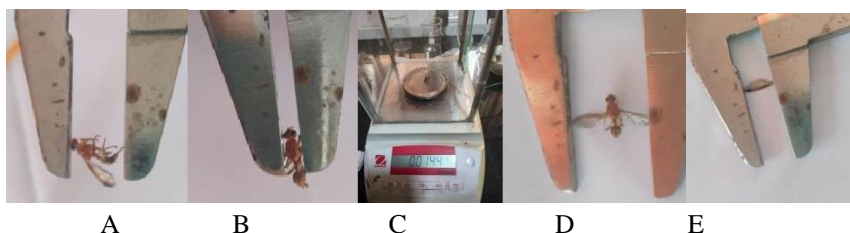


Figure 1. Measurement of adult of *Bactrocera minax* using Vernier Caliper (A: Body length, B: Body breadth, C: Body weight, D: Wingspan, E: Length of wing)

Management of Chinese citrus fly, *Bactrocera minax*: The management of Chinese citrus fly (*Bactrocera minax*) involves the use of protein-based baits to attract and control the population of these pests.

Preparation and application of protein bait solution: The solution used in our citrus orchard was prepared by combining water and bait in a 2:1 ratio. The protein bait employed, "Great Fruit Fly Bait", consisted of 25% protein hydrolysate and 0.1% abamectin. The initial step in preparation of solution formation involved counting a total of 93 citrus trees in the orchard. For the application of protein bait, 31 spots were selected initially, with an additional 5 spots chosen to ensure

coverage along the border lines. These spots were marked using colored ribbons tied to branches of selected trees. A mixture consisting of two parts water and one part protein bait was prepared, resulting in a 51 ml solution for each tree (17 ml of bait with 34 ml of water). For one spraying session, a total of 612 ml of protein bait was mixed with 1224 ml of water, totaling 1836 ml (bait + water) sprayed in each session.

12 spraying sessions were conducted weekly at Chhahari Retreat in Budanilkantha. Protein bait application was avoided during rainfall to prevent solution washout. Strategically placed small amounts of protein-based bait on the lower part of leaves ensured accessibility and minimized washout risks. Precautions and sanitation measures were diligently observed during application using a Knapsack sprayer.

Spray session: Weekly applications of spraying took place from 8th May 2023 to 24th July 2023. The specific dates and times of these spraying sessions are provided as follows (Table 1).

Table 1. Details for spraying session

Spray no.	Date of spray	Starting time of spray
1	8 th May 2023	1:00 pm
2	15 th May 2023	1:00 pm
3	22 nd May 2023	2:00 pm
4	29 th May 2023	1:00 pm
5	5 th June 2023	2:00 am
6	12 th June 2023	12:15 pm
7	19 th June 2023	1:20 pm
8	26 th June 2023	3:00 am
9	3 rd July 2023	1:00 pm
10	10 th July 2023	1:00 pm
11	17 th July 2023	2:00 pm
12	24 th July 2023	3:00 pm

Statistical analysis: The data collected were analyzed by using Microsoft excel 2013. The collected survey data were sorted and coded in Microsoft Excel. This organized data was then analyzed using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Measurement of pupae of Chinese citrus fly, *Bactrocera minax*

Table 2 shows the measurements of the pupae of Chinese citrus fly, ranged from 7.8 mm to 10.9 mm in length, with an average of 9.44 ± 0.10 mm. Pupal breadth

ranged from 3.3 mm to 5.8 mm, averaging 4.33 ± 0.09 mm. Pupae weighed between 0.018 gm and 0.084 gm. The average weight was recorded as 0.051 ± 0.002 gm as shown in table 2. Regmi et al., (2023) reported that the average pupal length was measured at 9.9 mm, the breadth at 4.42 mm, and the weight at 0.07 gm.

Table 2. Length, breadth and weight of pupae of Chinese citrus fly, *Bactrocera minax*

Length (mm)		Breadth (mm)		Weight (gm)	
Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE
7.8 - 10.9	9.44 ± 0.10	3.3 - 5.8	4.33 ± 0.09	0.018 - 0.084	0.051 ± 0.002

(n = 50), \pm SE= Standard Error

Adult emergence of Chinese citrus fly, *Bactrocera minax*

Adult emergence of the Chinese citrus fly was noted in the third week of April, with the highest frequency occurring in the first and second weeks of May. The date of adult emergence was recorded as April 17, 2023. These findings align with the observations of Regmi et al. (2023), who also reported that the emergence of the Chinese citrus fly took place in April and May.

Measurement of adult of Chinese citrus fly, *Bactrocera minax*

The adult Chinese citrus fly displayed a notable range in body dimensions as shown in Table 3, 4 and 5. Lengthwise, the span extended from 11.8 mm to 15.2 mm, with a mean length of 13.9 ± 0.20 mm of female and 9.7 mm to 13.7 mm with a mean length of 11.93 ± 0.33 mm of male. The breadth measurements revealed a variance from 3.4 mm to 4.3 mm, capturing an average breadth of 3.92 ± 0.06 mm of female and 2.8 mm to 3.6 mm with average breadth 3.18 ± 0.05 mm of male. Whereas, Gazmer et al. (2023) measured the adult male fruit flies body lengths ranging from 9.44 mm to 11.65 mm, with an average of 10.10 ± 0.25 mm and adult female fruit flies had an average body length of 11.28 ± 0.18 mm, with body lengths ranging from 10.34 mm to 11.88 mm. Also, the body width of adult female fruit flies is broader, measuring 3.60 ± 0.06 mm (ranging from 3.32 to 3.96 mm), which is notably different from adult males, whose body width is 3.09 ± 0.10 mm (ranging from 2.66 to 3.56 mm). As for the wingspan, the adults showcased a wingspan spectrum of 20.6 mm to 24.8 mm, averaging at 22.21 ± 0.23 mm of female and 19.7 mm to 23.3 mm averaging at 21.36 ± 0.30 mm of male. Delving into weight, the recorded range extended from 0.0041 gm to 0.0294 gm, aligning around an average weight of 0.0141 ± 0.002 gm of male and 0.0065 gm to 0.0308 gm with average weight of 0.0228 ± 0.001 gm of female. According to Adhikari et al., (2022), adult female fruit flies have a larger wingspan (ranging

from 21.55 mm to 25.45 mm) compared to adult males (ranging from 20.40 mm to 24.50 mm). The difference in wingspan between females and males is statistically significant with females having a mean difference of 0.71 mm. Diving further into wing specifics, the range of wing measurements spanned 8.1 mm to 10.3 mm, with an average wing length of 9.22 ± 0.16 mm of female and 7.7 mm to 10.1 mm with average wing length of 8.86 ± 0.16 mm of male. Generally, fruit flies have a wing length ranging from approximately 2 mm to 8 mm Gazmer et al. (2023), although specific species may fall outside this range. Another distinctive feature, the ovipositor length, varied from 5.5 mm to 3.2 mm, showing an average ovipositor length of 4.62 ± 0.14 mm. In a study by Adhikari et al. (2022), it was reported that the typical length of the ovipositor in female *Bactrocera minax* was approximately 4.52 mm. This comprehensive insight into the varied dimensions of the adult Chinese citrus fly provides a holistic understanding of its morphological characteristics.

Table 3. Body length, breadth and weight of adult Chinese citrus fly, *Bactrocera minax*

Sex	Length (mm)		Breadth (mm)		Weight (gm)	
	Range	Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE
Male (n=16)	9.7 - 13.7	11.93 ± 0.33	2.8 - 3.6	3.18 ± 0.05	0.0041 - 0.0294	0.0141 ± 0.002
Female (n=19)	11.8 - 15.2	13.9 ± 0.20	3.4 - 4.3	3.92 ± 0.06	0.0065 - 0.0308	0.0228 ± 0.001

Table 4. Wingspan and length of wing of adult Chinese citrus fly, *Bactrocera minax*

Sex	Wingspan (mm)		Length of wing (mm)	
	Range	Mean \pm SE	Range	Mean \pm SE
Male (n=16)	19.7 - 23.3	21.36 ± 0.30	7.7 - 10.1	8.86 ± 0.16
Female (n=19)	20.6 - 24.8	22.21 ± 0.23	8.1 - 10.3	9.22 ± 0.16

Assessment of Chinese citrus fly, *Bactrocera minax* management using protein bait in 2023

A significant decrease in fruit loss was achieved, dropping to 1.24% from the previously recorded 27.68% during the 2023 harvest season. This considerable reduction, amounting to a notable 26.44% decrease compared to 2022 ($p < 0.00$), was successfully realized as shown in Table 6. It is clear that the effect of Area-Wide Control Program of Chinese citrus fly using the protein bait in citrus orchard contributed a reduction in mean fruit damage. Similar result was published by Adhikari et al., 2021 entitled “Area-Wide Control Program in management of

Chinese citrus fly, *Bactrocera minax* (Enderlein) (Diptera: Tephritidae), in citrus orchards, Sindhuli, Nepal”.

Table 5. Length of ovipositor of adult Chinese citrus fly, *Bactrocera minax* (Female)

Length of ovipositor (mm) (n = 19)	
Range	3.2 - 5.5
Mean \pm SE	4.62 \pm 0.62

Table 6. Assessment of Chinese citrus fly, *Bactrocera minax* management using protein bait

Particulars	Fruit loss due to <i>B. minax</i> maggots' infestation in 2021	Fruit loss due to <i>B. minax</i> maggots' infestation in 2022	Fruit loss due to <i>B. minax</i> maggots' infestation in 2023
Mean fruit damage (%)	86.80	27.68	1.24
Variance	34.08	16.31	8.79
Observations	25.00	25.00	25.00
Pearson Correlation		0.11	-0.03
df		24.00	24.00
t Stat		39.63	-25.98
t-test for mean fruit damage % P(T<=t) one-tail		0.00	0.00
t Critical one-tail		1.71	1.71
t-test for mean fruit damage % P(T<=t) two-tail		0.00	0.00
t Critical two-tail		2.06	2.0

Source: Data of 2021 and 2022 (Thapa et al., 2023)

CONCLUSION

The morphometrics study highlighted notable morphological differences between male and female *Bactrocera minax*. Females were consistently larger and heavier than males. The implementation of an Area-wide Control Program (AWCP) using a lethal protein bait led to a remarkable reduction in fruit loss, decreasing from 27.68% in 2022 to just 1.24% in 2023. This substantial 26.44% reduction illustrates the effectiveness of the control measures and demonstrates their significant impact on reducing fruit loss.

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