RESEARCH ARTICLE

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EFFECT OF ALTITUDE ON ADULT EMERGENCE, PUPAL MORTALITY AND ADULT SEX RATIO OF CHINESE CITRUS FLY, *Bactrocera minax* (ENDERLEIN) (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Problems of Chinese Citrus Fly (CCF), Bactrocera minax (Enderlein) in citrus orchards have been increasing in Nepal. The reason behind the failure in CCF control is the lack of a clear and thorough understanding of the adult emergence period in different altitudes. Therefore, the pupae of CCF were collected from the soil below the infested sweet orange trees in Sunapati Rural Municipality, Ramechhap, Nepal and reared in containers of height 5 cm and diameter 6 cm (10 pupae per container) in randomized complete block design selecting different locations as treatments, viz. 1247 masl (Bethan), 1354 masl (Nagsiwa), 1443 masl (Aarukharka), 1561 masl (Sadi), 1650 masl (Dimipokhari) replicating four times. Early peak emergences of adult CCF (four weeks earlier in 4th week of April) occurred at lower altitudes as compared to the higher altitudes (in 4th week of May), where peak-emergence was recorded. Male: female ratio (range 1.05-1.37) did not differ significantly at different altitudes, while pupal mortality (25%) was found to be the highest in upper elevations. The study concludes that fly management strategy should be made according to the date of emergence at least two weeks earlier at lower elevations than in higher elevations.

1. INTRODUCTION

Citrus is the most popular and widely grown fruit crop in more than 140 countries of the world (FAO, 2021). In Nepal, citrus is cultivated in 50,235 ha with 32,188 ha productive area and 9.67 mt/ha productivity (MoALD, 2022). Mandarin (*Citrus reticulata*) occupies the first position in terms of area and production followed by sweet orange (*C. sinensis*) in terms of production, whereas lime in terms of area in Nepal (MoALD, 2020). Despite the well-accepted value and importance of citrus fruits in terms of income and climatic suitability, the production of citrus fruits is decreasing year after year. It is due to traditional methods of crop management, marginal production, legal and institutional constraints, inadequate irrigation, biotic and abiotic stresses and the major one is early fruit drop due to attack of the fruit flies (Gautam *et al*., 2020). Citrus fruits are depleted by fruit flies quantitatively and qualitatively (Adhikari, Tiwari and Joshi, 2018). Plant clinic data available in Plant-wise Online Management System (POMS) in 2016 highlighted the problems of fruit flies in vegetables, Cucurbitaceae and Solanaceae families (Adhikari, Thapa, Joshi, Pandit and Sharma, 2020). It not only causes yield loss by fruit dropping, but also limits the export trade of citrus fruits from Nepal because of the phyto-sanitary restrictions due to the fruit fly problems in the citrus orchards.

The world fauna of Tephritidae comprises nearly 4,716 species under 492 genera (Pape, Blagoderov and Mostovski, 2011). There are 27 species of fruit flies recorded in Nepal (Adhikari, Thapa, Joshi and Du, 2022). Out of them, citrus fruit loss in Nepal is commonly caused by Chinese citrus fly (CCF), *Bactrocera minax* (Enderlein), which has become a serious threat in citrus production since 2014 in central hilly districts (Sindhuli and Ramechhap) with up to 97% fruit loss (Adhikari, Tiwari and Joshi, 2018). The reasons behind the failure in fly control are a lack of clear and thorough understanding of pest biology, population dynamics, agro-ecosystem and climatic patterns (Adhikari, Thapa, Joshi, Liang and Du, 2020). The overwintering pupa of

CCF remains in the soil of depth 3-7 cm (Wu, Zhang and Liang, 2008) for 5 to 7 months (Sharma and Dahal, 2020). These species have the capacity to withstand high moisture content by lowering their respiration rate (Li *et al*., 2019). The adult emerges from the soil in April/May which reaches to its peak in June / July and starts to decline in August (Sharma and Dahal, 2020). The pest, *B. minax* requires one year for completing its life cycle. This study presents the relationship between adult emergence, fly mortality and sex ratio in relation to altitude variations, so that management measures could be made accordingly.

2. MATERIALS AND METHODS

2.1 Site of study

Figure 1. Map of Nepal showing Ramechhap and research site of Sunapati Rural Municipality

Figure 2. Placement of pupae in the container

The experiment was done in randomized complete block design (RCBD) in Sunapati Rural Municipality, Ramechhap, Nepal from April to June, 2021. Pupae of citrus fruit fly were collected from the soil below the infested trees of sweet orange on 10th March, 2021 from three different locations (Bethan, Aarukharka and Dimipokhari), and sheltered in small cylindrical containers of height 5 cm and diameter 6 cm containing slightly moist sandy soil below 1 cm from the top of the container at different altitudes to see its date of emergence. For this, a total of 200 pupae were collected. These pupae were reared at five different elevations (treatments) on 2nd April, 2021, viz. 1247 masl (Bethan), 1354 masl (Nagsiwa), 1443 masl (Aarukharka), 1561 masl (Sadi), 1650 masl (Dimipokhari) using 40 pupae at each elevation replicating 4 times in 4 containers (10 pupae per container). Date of emergence, male-to-female ratio and pupal mortality percentage of CCF were calculated.

2.2 Adult Sex Ratio (Male: female)

Male: female ratio was calculated by dividing emerged number of male adult fly by emerged number of female fly.

$$\text{Male:female ratio} = \frac{\text{Emerged number of male fly}}{\text{Emerged number of female fly}}$$

2.3 Mortality Percentage of Pupae

Pupal mortality percentage was calculated as follows:

$$\text{Pupal mortality percentage} = \frac{\text{Number of dead pupae inside rearing container}}{\text{total number of reared pupae}} \times 100\,\%$$

2.4 Statistical Analysis

The data were recorded in an Excel worksheet and subjected to Genstat® 15th edition for Analysis of Variance (ANOVA). Before performing the ANOVA, data were transformed through square root transformation i.e. $\sqrt{(x+0.5)}$ to reduce the heterogeneity of variance. Duncan's Multiple Range Test (DMRT) at a 5% level of significance was used for mean comparison (Gomez and Gomez, 1984).

3. RESULTS AND DISCUSSION

The emergence of adult CCF differed significantly at different altitudes on different weeks/months of the year except for 2nd week of May and June (Table 1). The higher mean numbers of CCF emerged during the 2nd, 3rd and 4th week of April at lower altitudes (1247 masl), whereas higher adult CCF emerged only during the 4th week of May and 1st week of June at higher altitude (1650 masl). At lower altitude, most of adults emerged during April and at higher altitude, most of them emerged during May. Similar results were found in China (Xia, Ma, Hou and Ouyang, 2018) in which adult flies of CCF emerged from March to April depending upon local temperature. A similar result was also observed by NCRP, Paripatle, Dhankuta Nepal (NCRP, 2014). The highest emergence at lower altitudes might be due to the adequate and sufficient temperature for pupal eclosion. These findings are supported by Adhikari, Thapa, Joshi, Du and GC (2021) in which they found the longer diapause intensity of 165.5 days and 164.2 days at higher altitudes as compared to shorter diapause intensity of 143.5 and 141.3 days at lower altitudes for sweet orange and lemon, respectively. Acharya and Adhikari (2019) found the delayed in emergence of Chinese citrus fly with increase in altitude due to lower temperature at higher altitudes. Xiaofei and Hui (2009) conducted a study to find out the effect of temperature on development and survival of *B. correcta* and found that the higher temperature was the most conducive for reaching early maturity. Gautam *et al*. (2020) also found early emergence of adult Chinese citrus fly at lower elevation as compared to upper elevation. But Dong *et al*. (2013) found the termination of pupal diapause faster at long and more chilling temperature.

Adult sex ratio of CCF was found to be almost similar for all altitudes (Table 2). However, the ratio was greater than 1, which means the emergence of pupae was dominated by the male population. This result showed that male-to-female ratio was similar regardless of variation in altitudes. Regmi *et al*. (2023) also found non-significant differences among sex ratios at different altitudes. However, Chauhan *et al*. (2019) found the highest male-to-female ratio at a lower altitude of the range 1400-1474 masl. Pupal mortality was found to be the highest for upper elevations as compared to lower altitudes (Figure 3). This might be the result of high chilling temperatures during winter months.

Table 1. Effect of different altitudes on number of adult emergences of CCF, *B. minax* in different dates in Ramechhap district, Nepal

Altitude range (masl)	1st week Apr	2nd week Apr	3rd week Apr	4th week Apr	1st week May	2nd week May	3rd week May	4th week May	1st week Jun	2nd week Jun
1247	0.5	2.62	3.46	3.49	1.71	0.92	0.5	0.5	0.5	0.5
(Bethan)	(0.71^{b})	(1.62^{a})	(1.86^{a})	(1.87^{a})	(1.31^{b})	(0.96)	(0.71^{b})	(0.71^{c})	(0.71^{b})	(0.71)
1354	0.5	0.5	1.39	3.13	3.46	2.10	0.92	0.5	0.5	0.5
(Nagsiwa)	(0.71^{b})	(0.71^{b})	(1.18^{bc})	(1.77^{a})	(1.86^{a})	(1.45)	(0.96^{b})	(0.71^{c})	(0.71^{b})	(0.71)
1443	1.18	0.69	1.96	2.92	1.61	1.82	1.10	0.69	0.5	0.5
(Aarukharka)	(1.09^{a})	(0.83^{b})	(1.4^{b})	(1.71^{a})	(1.27^{b})	(1.35)	(1.05^{b})	(0.83^{bc})	(0.71^{b})	(0.71)
1561	0.5	0.5	0.5	1.10	0.5	1.61	5.15	1.56	1.71	0.5
(Sadi)	(0.71^{b})	(0.71^{b})	(0.71^{b})	(1.05^{b})	(0.71^{c})	(1.27)	(2.27^{a})	(1.25^{ab})	(1.31^{a})	(0.71)
1650	0.5	0.5	0.92	0.5	1.49	1.61	1.39	2.43	1.39	0.84
(Dimipokhari)	(0.71^{b})	(0.71^{b})	(0.96^{cd})	(0.71^{b})	(1.22^{b})	(1.27)	(1.18^{b})	(1.56^{a})	(1.18^{a})	(0.92)
SEM (±)	0.11	0.21	0.25	0.28	0.21	0.34	0.31	0.28	0.18	0.19
$LSD_{0.05}$	0.18	0.34	0.39	0.44	0.31	ns	0.48	0.44	0.27	ns
CV%	14.7	23.8	20.7	20.2	16.2	27.6	25.2	28.2	19.3	26
Grand mean	0.78	0.92	1.22	1.42	1.27	1.26	1.24	1.01	0.92	0.75
F value	0.001	<0.001	<0.001	<0.001	<0.001	0.41	<0.001	0.004	<0.001	0.44
	***	***	***	***	***		***	**	***	

CV=Coefficient of variation, Means followed by the same letter in a column are not significantly different by DMRT at 5%, ** significant at 1% level of significance, *** significant at 0.1% level of significance, ns non-significant. Figures in parenthesis indicate data transformed to $\sqrt{(x + 0.5)}$, SEM= Standard error of the mean, LSD0.05= Least significant difference at 5% level of significance.

Table 2. Altitude-wise adult sex ratio of CCF, *B. minax* in Sunapati Rural Municipality, Ramechhap district, Nepal

Location	Altitude (masl)	Male: female ratio
Bethan	1247	1.25
Nagsiwa	1354	1.08
Aarukharka	1443	1.13
Sadi	1561	1.37
Dimipokhari	1650	1.05

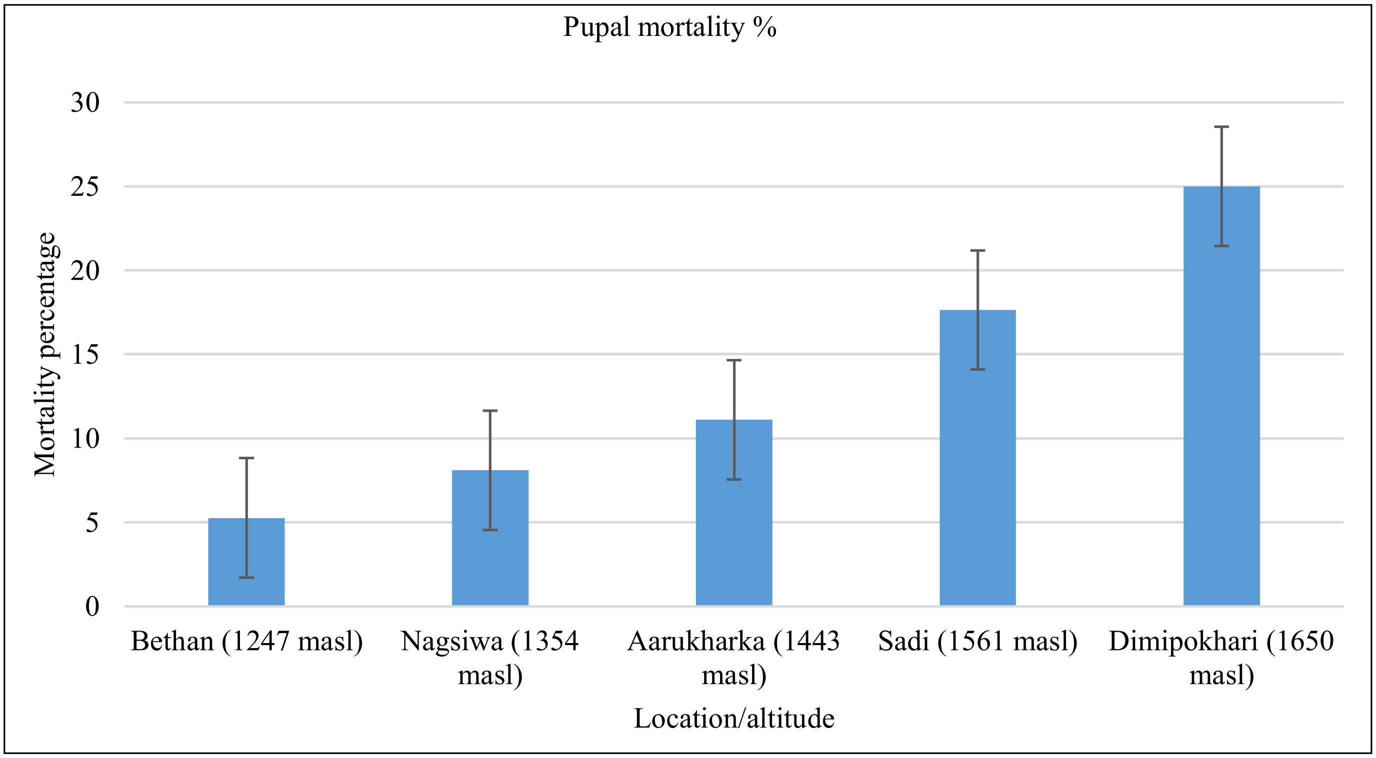

Figure 3. Average pupal mortality (percentage-wise) ± SEM of CCF, *B. minax* at different altitudes in Sunapati Rural Municipality, Ramechhap district, Nepal

4. CONCLUSION

The adult sex ratio of CCF, *B. minax* is independent of various altitudes, whereas pupal mortality is found to be the highest in upper elevations. Early emergence of adult fly occurred at lower altitudes as compared to the late emergence at higher altitudes. Hence, the effective management of CCF, *B. minax* is based on the date of adult emergence at different altitudes. The present finding indicates that management of CCF, *B. minax* should be practiced in the citrus orchard at least by two weeks earlier at lower altitudes than at higher elevations.

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