

Assessment of Pest Severity and Biological Parameters of *Bactrocera minax* in Sweet Orange Orchards in Central Nepal

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Abstract—Chinese Citrus fly, *Bactrocera*(*Tetracus*) *minax*(Enderlein), univoltine fruit fly species, is a serious insect pest in Nepal, Bhutan, China, India causing 100% fruit drop in sweet orange (*Citrus sinensis* L.) orchards in severe case. Four elevation ranges: 1400-1474masl, 1475-1549masl, 1550-1624masl and >1624masl of Ramechhap district were taken for the study of severity of infestation by this fly species in November 2018. A subsequent rearing was conducted at Agriculture and Forestry University, Chitwan, Nepal upto April 2019 to assess various developmental parameters of *Bactrocera minax* starting from larval stage in infested sweet oranges to the adult flies. Elevation range had the most significant effect ($P < 0.05$) on pest severity (2017/18). Pest severity had strong relationship on elevation of orchards ($R^2 = 0.6638$). Maximum pest severity (37.12%) was found in 1550-1624masl and minimum (2.90%) in 1400-1474masl. Maximum mean maggots/fruit (6.40 ± 1.25) at 1550-1624masl and minimum (3.95 ± 0.92) at 1475-1549masl were recorded. Post-pupal mortality was higher than pre-pupal mortality. Maximum pre-pupal mortality ($11.13 \pm 5.24\%$) at >1624masl and the minimum ($2.08 \pm 1.46\%$) at 1550-1624masl were recorded while 1475-1549masl and 1400-1474masl had the respective minimum ($25.81 \pm 7.59\%$) and maximum ($36.08 \pm 9.17\%$) post-pupal mortality. Most adult flies emerged by 2nd week of March lasting 115 days for adult eclosion. Sex ratio (male: female) was maximum (2.5) at 1400-1474masl and minimum (1.2) at >1624masl. Without feeding an adult fly survived upto 3 days. It can be speculated that besides other meteorological factors, elevation affects geographical distribution of fly and its subsequent biological parameters.

Keywords—*Bactrocera minax*, fruit drop, Nepal, sweet orange.

I. INTRODUCTION

Chinese Citrus fly, *Bactrocera* (*Tetracus*) *minax* (Enderlein), a dipteran belonging to the sub-family Dacinae of the family Tephritidae is one of the most destructive insect pests causing huge loss of Sweet orange (*Citrus sinensis* L.) upto 100% in severe case and more than 50 % fruit drop of mandarin (*Citrus reticulata* Blanco) (Dorjiet al., 2006). It was recognized as major limiter of commercial citrus, especially in China for more than a half century (Wang & Luo, 1995). The infestation of the fly is quite common in mid and high hills in citrus orchards above 1100m (Van Schoubroeck, 1999). This fruit fly is unique of its sub-family, Dacinae being univoltine and having larval host range restricted in wild and cultivated citrus species (Allwood et al., 1999).

In past few years, major sweet orange (*Citrus sinensis* L.) producer districts of Nepal like, Ramechhap, Sindhuli, Dolakha have faced an unprecedented decline in citrus production due to the infestation by *B. minax* (myRepublica, 2018). Adhikari & Joshi, in 2018 reported that fruit loss due to the infestation by Chinese Citrus Fly (*Bactrocera minax*) is becoming devastating in Sindhuli and Ramechhap district since 2014. In 2019, Adhikari et al. reported that the pest is even suspected to Lamjung district, western Nepal. The pest was first misidentified as *B. tsuneonis* Miyaka in Sweet orange orchard in Helambu, Sindhupalchowk district in December 1984 (Adhikari et al., 2019). The peculiarity of *B. minax* among its genus is that the fly is univoltine, oligophagous exclusively feeding on citrus plants with the

long sized cold tolerant larvae and adult with 16 mm and 24 mm respectively (Xia *et al.*, 2018). Similar result was reported by Adhikari *et al.*, in 2019 from sweet orange orchards of Sindhuli district, Nepal.

Due to little research on phenology and ecology of *B. minax*, until 2008, it had been wrongly identified as *Bactrocera dorsalis* for decade, in eastern Nepal that led to the ineffective management of Chinese Citrus fly by Male Annihilation Technique using Methyl Eugenol (ME) and Cue Lure (CL) trap (NCRP, 2014). However, this species can be attracted much in green-yellow colored mimic sphere for the study (Drew *et al.*, 2006). Also, the research works on *B. minax* is exclusively published in Chinese journals and in Chinese language, which led to the failing of a comprehensive study on this pest outside China especially for its monitoring, detection and phytosanitary measures (Xia *et al.*, 2018).

Therefore, the need was to make some more focused recommendation on future management of this devastating pest in Nepal and study the severity of pest infestation. Specifically, this experiment was carried out to know when the adult emergence occurs at various elevations in Ramechhap district and for how long pupal overwintering lasts so that effective control measures could be applied with the observed information. Therefore, based on orchard observation: i) severity of infestation by fly ii) geographical distribution of maggots population per fruit at various elevations would be helpful to prioritize the management procedures at respective places. Various developmental parameters of this fly, such followings: i) pre-pupal mortality(larvae to pupa) ii) post-pupal mortality(pupae to adult) iii) larvae to adult mortality iv) pupation duration v)adult emergence season vi) post emergence death period of *B. minax* would provide information on future study on taking under control of the insect and to apply efficient management measures.

II. MATERIALS & METHODS

This research consists two parts: the first, field survey on various orchards at different elevations, in Ramechhap district, Nepal, the second, lab experiments at Entomology Laboratory of Agriculture and Forestry University, Chitwan, Nepal.

2.1. Site of Orchards

In November 2018, random sweet orange orchards, under farmers' practice at 4 different the elevation ranges: 1400-1474masl, 1475-1549masl, 1550-1624masl and >1624masl were selected. The elevation was determined by respective

co-ordinates of longitude and latitude recorded by Google map (Elevation finder, 2018).

2.2. Experimental Designs:

The experiment was conducted in CRD with 4 treatments and 4 replications. Four ranges of elevation viz. 1400-1474masl, 1475-1549masl, 1550-1624masl and >1624masl were taken as treatments.

2.3. Rearing Condition

The experiment to assess the pupation and adult emergence of *Bactrocera minax* was conducted on fruit fly maggots infested sweet orange fruits at Entomology laboratory of Agriculture and Forestry University, Chitwan, Nepal from Nov. 2018 to April 2019. The mean maximum and minimum temperatures recorded were 19.22°C and 16.98°C with mean relative humidity 59.33%. Eighty random sweet orange fruits were collected from the orchards and fruits were kept individually in plastic case of 30cm × 15 cm size, considering it to be the day 0 after fruit fall, provided with 18 cm thick sandy loam soil having 13.80 % moisture for facilitating pupation and covered with muslin cloth for observation of developmental studies of the flies. Fruits were dissected after 7 days as the 1st and maximum pupation i.e. 22.6% occurs till 7 days (NCRP, 2014) and counted the no. of maggots remaining in the fruits to calculate the no. of matured 3rd instar larvae in week that would go into the soil for overwintering.

We assumed that the total pupae (live and dead) would be equal to the no. of maggots in the infested fruits and no. of matured 3rd instar larvae that went for puparia development in soil would be the total maggots less the maggots remaining in the fruit. The pupation count for the second time was done on 1st Jan 2019 after 5 weeks of the fruit fall, sieving the soil and consciously. Then after, net was used to cover the opening of each rearing case to avoid the possible escape of the adult flies. Emerged adult fruit flies were identified according to the morphological characters given by Adhikari & Joshi, 2019 and recorded the sex ratio (male: female). Also, 5 pairs of male and female flies were separately kept in the separate vials for post emergence death observation without artificial feed.

The developmental periods of maggots, pupae and post emergence death of adult without feeding, mortality rate of each stage were recorded and subjected to the statistical analysis.

2.4. Statistical Analysis

Data compilation was carried out using MS Excel and analyzed using R (Version 6.3.1). Data was transformed

using square root transformation $\sqrt{(x+0)}$. Other biological parameters were represented in graphical and tabular forms.

III. RESULTS AND DISCUSSION

3.1. Pest Severity:

Table1: Effect of elevation ranges on Severity of infestation (2017/18) by *Bactrocera minax* in Ramechhap district, 2018

Elevation ranges	Pest Severity (%)
1400-1474masl	2.087 ^b
1475-1549masl	2.075 ^b
1550-1624masl	37.125 ^a
>1624masl	23.5975 ^a

SEM 1.6914
Grand Mean 3.377175

[Means followed by same letter(s) in a column are not significantly different at 5% level of significance in DMRT test. SEM: Standard Error of Mean, *** is significant at P]

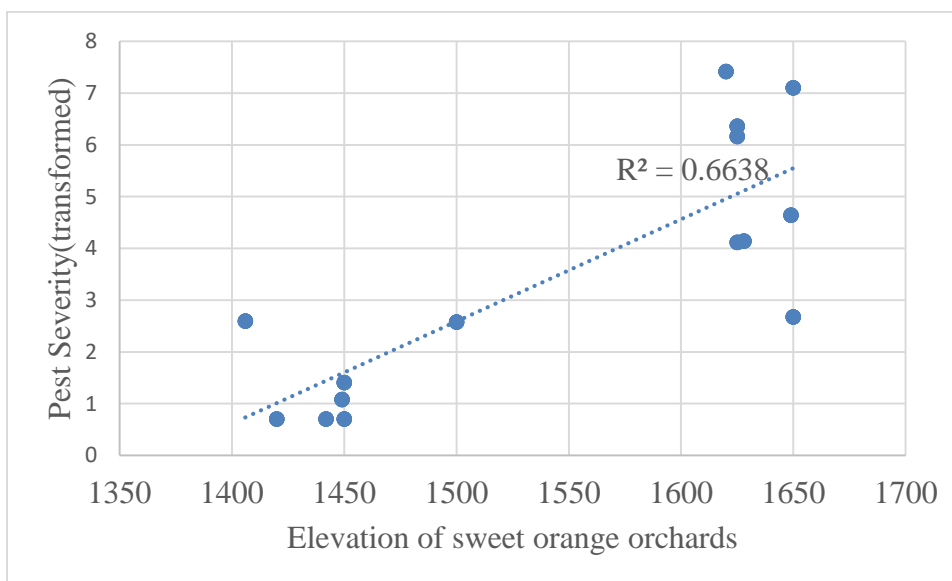


Fig. 1. Relationship between severity of infestation (2017/18) by *Bactrocera minax* and elevation of sweet orange orchards in Ramechhap district, 2018

As there is no effective method of trapping adults of *Bactrocera minax*, Xia *et al.*, in 2018 suggested that fruit infestation by the fly is the only reliable indicator to measure the pest severity. The maximum pest severity was found at 1550-1624masl and the minimum pest severity at 1475-1549 masl. The result was consistent with the findings reported by Xia *et al.*, 2018 in China. They found the most severe cases of fruit fly lied in moderate range of citrus growing elevation.

Besides, farmers’ management practice like spraying protein bait, collection and removal of dropped fruits within few hours of fruit drop decrease the pest severity the following season (Van Schoubroeck, 1999). The value of coefficient of determination between pest severity with elevation range ($R^2 = 0.6638$) showed that besides other factors, elevation alone had 66.38% contribution in pest severity.

3.2. No. of maggots per infested fruit:

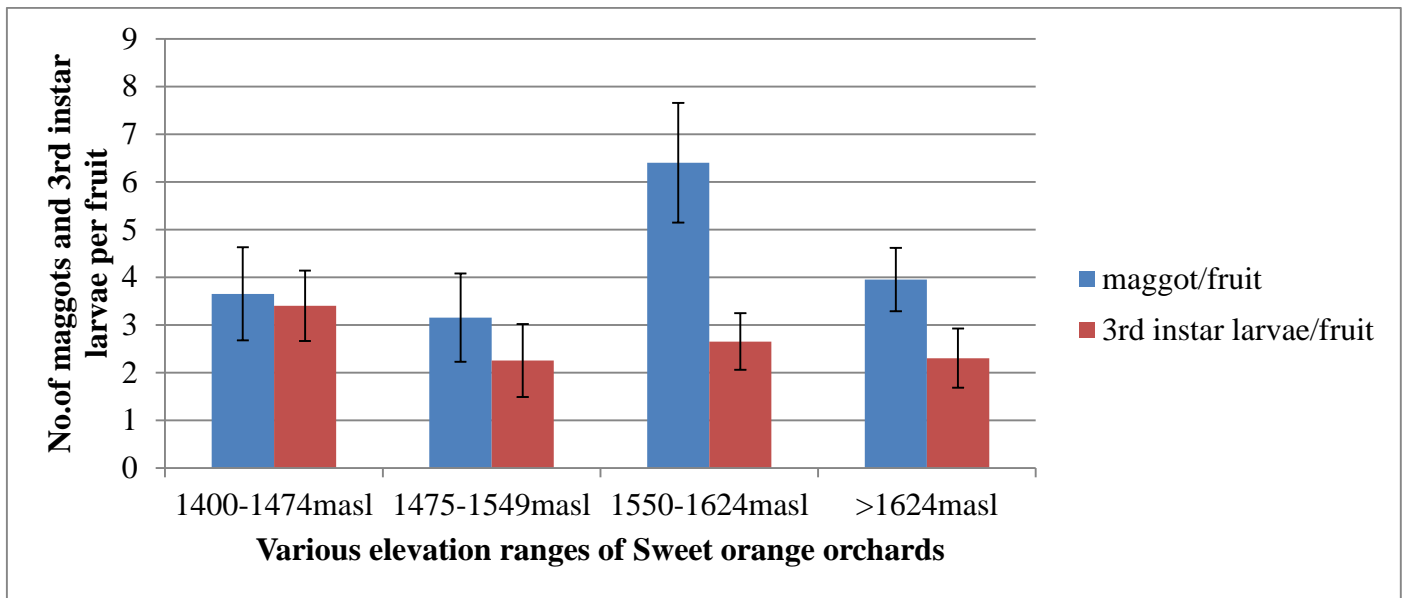


Fig. 2. Mean maggots per fruit and mean no. of 3rd instar larvae of *Bactrocera minax* on 7th day of fruit drop in Ramechhap district, 2018

The result showed that maggot density was the maximum i.e. 6.40 ± 1.25 at elevation 1550-1624masl and minimum i.e. 3.15 ± 0.92 at 1475-1549masl.

3.3. Pupation behavior of maggots:

The first and maximum pupation i.e. 22.6% occurs till 7th day of fruit fall (NCRP, 2014). Only the matured 3rd instar larvae

move into soil. The study depicted that till 7 days after fruit fall, maximum 3.4 ± 0.59 matured 3rd instar larvae at 1550-1624masl and went to soil for the pupation while minimum pupation 2.25 ± 0.59 began in >1624masl and intermediate proportion of pupation at 1475-1549 masl and 1400-1474masl i.e. 2.65 ± 0.76 and 2.3 ± 0.73 respectively.

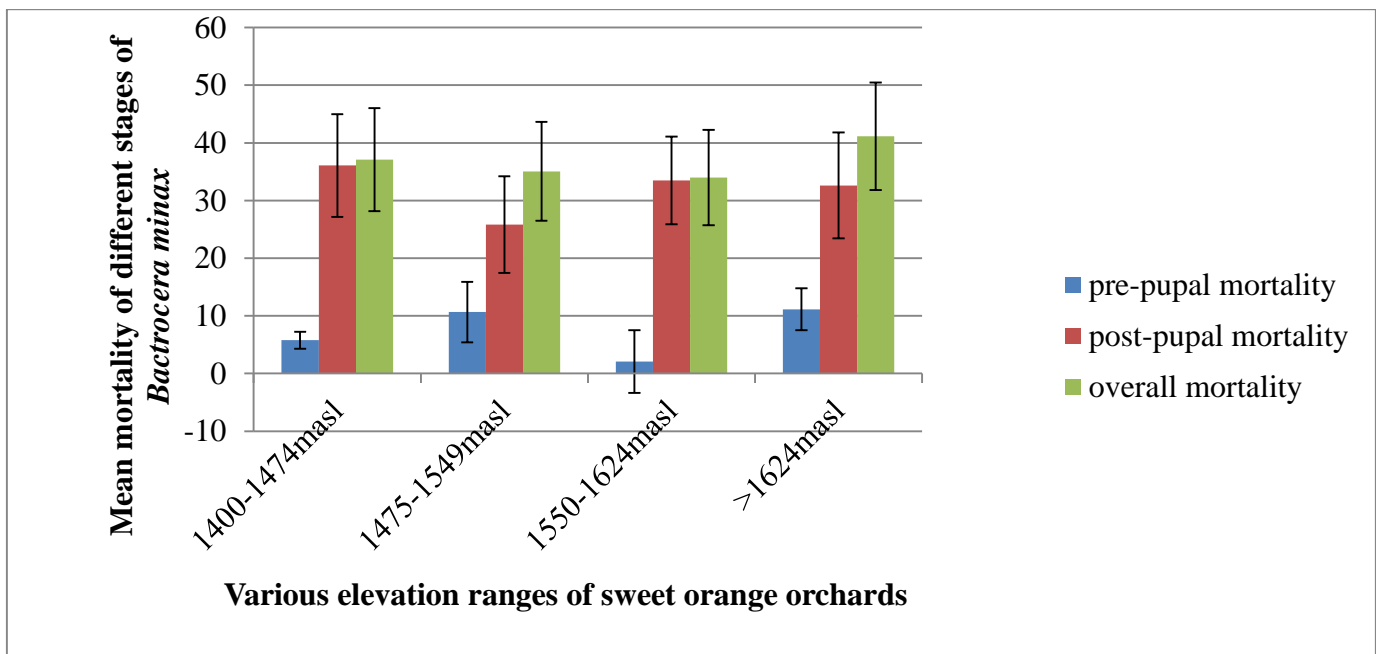


Fig. 3. Mean mortality (\pm SEM) of different stages of *Bactrocera minax* observed at Agriculture and Forestry University, Chitwan in 2019

3.4. Mortality of maggots:

The maximum pre-pupal mortality was found to be 11.13 ± 5.24 % in >1624 masl while 1550-1624masl had the minimum pre-pupal mortality with 2.08 ± 1.46 %. 1475-1549 masl and 1400-1474masl had as high as 10.67 ± 5.44 % and 5.77 ± 3.63 % respectively. As *B. minax* is a cold adapted species (Xia *et al.*, 2018), it can be speculated that during rearing at comparatively higher lab temperature, the larvae from higher elevation was much impaired than the larvae from the lower elevation that governed the pre-pupal mortality.

3.5. Mortality of pupae:

The study conferred that post-pupal mortality was higher to the pre-pupal mortality. Further, 1400-1474masl and 1475-1549masl had the respective highest and the lowest post-pupal mortality of the fly as 36.08 ± 9.1 and 25.81 ± 7.59 .

3.6. Overall mortality of larvae:

The range of mortality of larvae to adult was seen with almost in similar patterns in each elevation ranges ranging

from 34 ± 8.94 % at 1550-1624masl to 41.14 ± 8.59 % in >1624 . Similarly, 35.06 ± 8.27 % and 37.10 ± 9.32 % mortality rate was found in respective at 1475-1549masl and 1400-1474masl.

Due to same lab temperature, pupal mortality was affected much with other factor like soil moisture than temperature. Since the initial soil moisture was only 13.6%, it might be detrimental factor for pupal survival in soil. According to the study by Hulthen & Clarke, 2006 in other species of fruit fly, *Bactrocera tryoni*, 85% pupal mortality was observed in dry soil (0% moisture). Another study by Li *et al.*, in 2019 suggested that dry soil (soil with lower moisture content) is detrimental to *B. minax* pupae than the soil with high moisture content. They concluded that too much water is lost from pupae and can't obtain water from outside when soil moisture is low. Also, Dong *et al.* in 2013 reported that pupal mortality tends to surge more than 70% when pupae are exposed to natural chilling more than 3 months.

3.7. Measurement of pupal eclosion days:

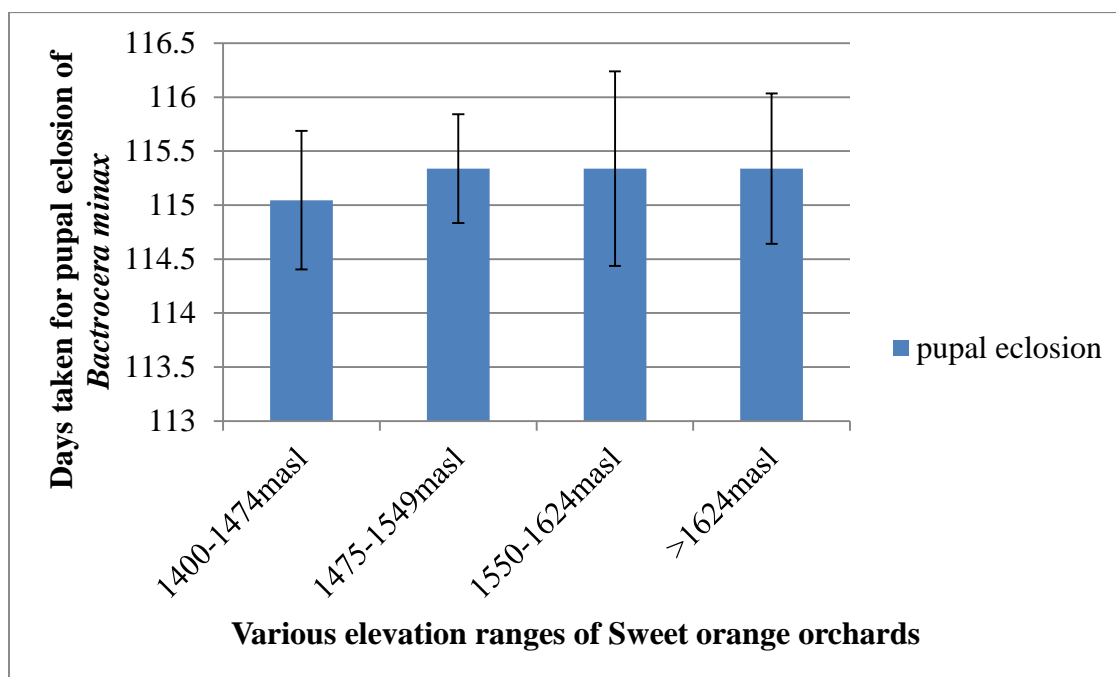


Fig. 4. Days (\pm SEM) for pupal eclosion of *Bactrocera minax* reared at Agriculture and Forestry University in 2019

Considering the first day of experiment i.e. 22nd Nov.2018 to be the day 0 after fruit fall and the 7th day after fruit fall to be the beginning of pupation we calculated the eclosion days of pupae. It took 115.33 ± 0.89 days in 1475-1549masl while 115.33 ± 0.69 in 1400-1474masl for pupal eclosion. Also, 1550-1624masl and 6 had the nearly matching pupation

duration with 115.04 ± 0.64 and 115.33 ± 0.50 days respectively.

Due to the same meteorological condition of the lab, there was no relative difference between the pupation duration between elevation ranges.

3.8. Adult fruit fly emerged and sex ratio:

Table 2: Mean numbers (\pm SEM) of adult, male and female *Bactrocera minax* and their sex ratio (male:female) observed at Agriculture and Forestry University in 2019

Elevation ranges	Mean male fly emergence/fruit	Mean female fly emergence/fruit	Sex Ratio(M: F)	Mean adult emergence/fruit
1400-1474masl	1.15 \pm 0.42	0.55 \pm 0.28	2.09	2.15 \pm 0.51
1475-1549masl	1.15 \pm 0.42	0.75 \pm 0.39	1.53	4.4 \pm 1.03
1550-1624masl	1.2 \pm 0.29	1 \pm 0.30	1.2	1.90 \pm 0.76
>1624masl	2.45 \pm 0.71	1.95 \pm 0.43	1.26	1.70 \pm 0.62

Emerged adult flies as well as male and female fly counts were highest at 1475-1549masl with 4.4 \pm 1.03, 2.45 \pm 0.71 and 1.95 \pm 0.43 respectively. Also, range 1550-1624masl had the minimum no. of adult count 1.7 \pm 0.62 with minimum no. of male 1.15 \pm 0.42 and female counts 0.55 \pm 0.28.

Further, sex ratio (male:female) of the fly was near unity, however male flies were found to be abundant compared to female. 1400-1474masl and 1550-1624masl had the respective highest male: female (sex ratio) i.e.2.09 and the lowest i.e. 1.20.

3.9. Adult emergence:

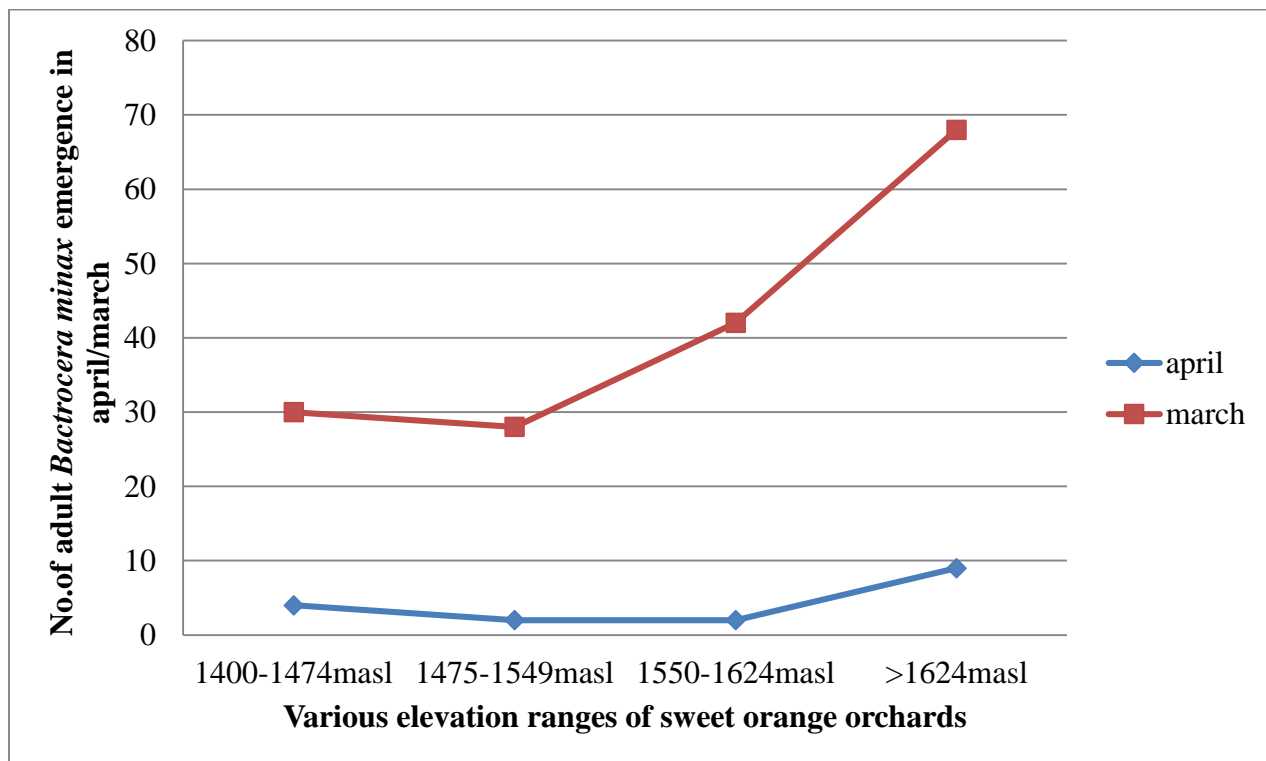


Fig. 5. Total number of adult *Bactrocera minax* emergence per month as observed at experimental set ups of various elevation ranges at Agriculture and Forestry University in 2019

It was observed that fly emerged in both March and April, however most fly emergence occurred in March and only little in April. In both March and April, >1624masl had the highest rate of emergence while lowest emergence, in March occurred in 1400-1474masl. Also, 1550-1624masl and 1475-1549masl had lower and comparatively equal state of adult

emergence in April. The first flies emerged on 17th March and the last flies on 4th April 2019.

The experiment showed that adult emergence started by 2nd week of March. The result is quite consistent with Dorjiet *al.*, in 2006 which reported that adult emerges by mid of March to Adult in Western Bhutan. In China, depending upon the

local temperature of provinces, adult flies emerge by March to April (Xia *et al.*, 2018). Similar case was reported by NCRP, Paripatle, Dhankuta Nepal (NCRP, 2014). Adhikari

et al., 2019 observed that March to April is the adult emergence season in Sindhuli, Nepal.

3.10. Measurement of Post-emergence death duration:

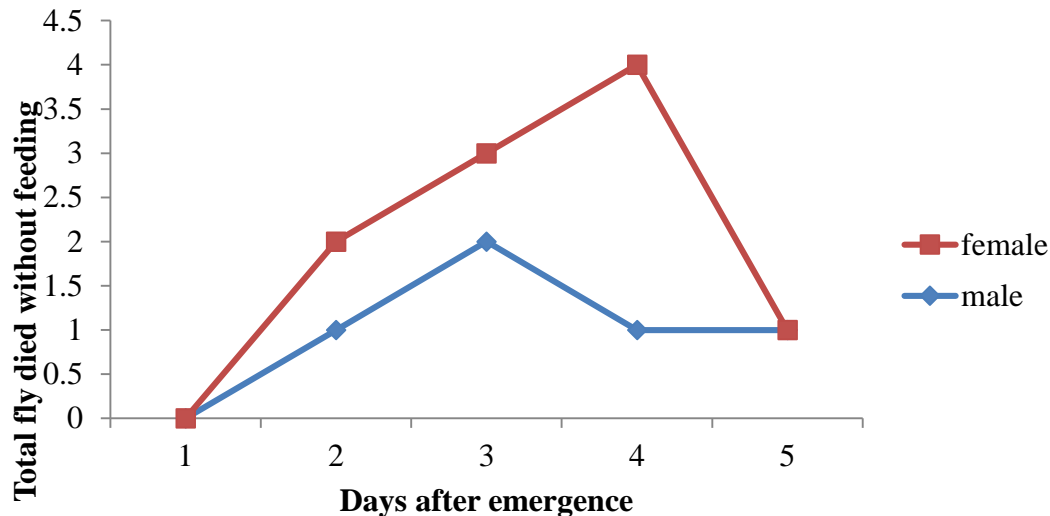


Fig. 6. Total post-emergence death interval of *Bactrocera minax* with no feeding at Entomology Laboratory, Agriculture and Forestry University, Chitwan, Nepal in 2019.

Further, there was no obvious difference in the periods of death interval of sexes after the emergence without giving any feeds. Compared with 5 flies of each sex, female flies died within 4 days after emergence while 1 male survived till 5th day. It was found that male died in 3.4 ± 0.50 and female in 3.4 ± 0.40 days after emergence.

IV. CONCLUSION

The *Bactrocera minax* is major pest of sweet orange in Ramechhap district of Nepal. Majority of sweet orange orchards in Ramechhap district lies at intermediate range of elevation i.e. 1550-1624masl, where the case of fly is most prevalent. In central mid hill like Ramechhap, management practices for adult fruit fly should be undertaken before 1st week of March as adult fly emergence initiates by 2nd week of March. In individual orchards, in Ramechhap district severity of infestation by Chinese Citrus fly ranges from 0% to 100% that resulted in almost nil fruits in harvest season. Clearing off the infested fruits and killing maggots as soon as fruits drop on orchards are crucial measures to decrease the severity of infestation by fly in the next production season. Further, location specific research on its phenology is suggested to bring forth effective control over this pest.

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