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Diapause intensity of Chinese citrus fly, *Bactrocera minax* (Enderlein) in Sindhuli, Nepal

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Abstract
Chinese citrus fly, *Bactrocera minax* (Enderlein) is a serious pest of citrus fruits in Nepal. Understanding the life stages and behavior of this pest is important to plan management strategy. The pupal stage of this pest completes in soil and exhibits overwintering as pupal diapause during the winter season. This study showed that days to adult emergence for the maggots from sweet orange and lemon differed significantly (≤0.05) between higher (Khaniyakharka 1341 masl) and lower altitude (Sindhulimadi 503 masl). The higher percentage of pupation from pre-pupae occurred at higher altitude, i.e. Khaniyakharka (91.50 ± 1.92) than at lower altitude, Sindhulimadi (86.50 ± 2.32), which was slightly higher in sweet orange (90.25 ± 2.06) than in lemon (87.75 ± 2.30). However, adult emergence was recorded at > 93% in both locations and citrus fruits. At higher altitudes, the diapause intensity was longer, 165.5 days and 164.2 days as compared to 143.5 and 141.3 days at a lower altitudes for sweet orange and lemon, respectively. Hence, the diapause intensity of the Chinese citrus fly was dependent on the prevailing temperature conditions at different locations.

Keywords: *Bactrocera minax*, citrus pest, diapause, emergence, Nepal

Introduction
Chinese citrus fly, *Bactrocera minax* (Enderlein) is one of the important insect pests of citrus fruits in Nepal (Adhikari and GC, 2020; Adhikari *et al.* 2020; Bhandari *et al.* 2017; Sharma *et al.* 2015) [1, 2, 3, 4]. In recent years, the pest is becoming a devastating pest of citrus orchards in the western parts of Nepal, after it's greater infestion in the eastern and central hilly districts of Nepal (Nayapatrika Daily, 2010) [5]**.** The pest is native to China and spread to Nepal via Bhutan and India (Adhikari *et al.* 2019; Acharya and Adhikari, 2019; Adhikari and Joshi, 2019) [6, 7, 8]. Protein bait based area-wide control program was initiated in the Sindhuli district in 2018 and it is gaining popularity in pest management in the other parts of the country.

This pest is unique because of its larger size and univoltine life cycle, with oligophagous feeding habit, exclusively feeding on citrus fruits: Rutaceae, and it is not attracted by the para-pheromone lure; neither cue lure, nor methyl eugenol (Adhikari and Joshi, 2018; Xia *et al.* 2018; Wang and Luo, 1995; Chen and Xie, 1955) [9, 10, 11, 12]. So, protein bait is commonly used to monitor and manage this pest (Yasuda *et al.* 1994; Adhikari *et al.* 2019) [13, 6]. The understanding of insect development and behavior during various life stages of this pest is crucial for its effective control. In this case, the location of orchards, weather conditions is also important in determining the development of the pest, of which the time of adult emergence is of vital importance for the timing of protein bait application. This paper highlights the study of rearing pupae from pre-pupae (matured maggots) and completion of pupation period at two contrast elevations i.e. higher (Khaniyakharka, 1341 masl) and the lower (Sindhulimadi, 503 masl) altitude of Sindhuli district.

Materials and Methods
Site of study
This study was conducted from early November 2019 to late April 2020 in the laboratory room at lower altitude Sindhulimadi (latitude: $27^0$12.777' N, longitude: $85^0$54.858' E, altitude: 503 masl) at Prime Minister Agriculture Modernization Project (PMAMP), Project Implementation Unit (PIU), Sindhuli Contact Office and higher altitude Khaniyakharka (latitude: $27^0$17.145' N, longitude: $85^0$58.675' E, altitude: 1341 masl) at Plant Protection Laboratory of PMAMP, PIU, Golanjor-5, Sindhuli in Sindhuli district, Bagmati Province of Nepal (Figure 1). The GPSMAP 62s (garmin.com/en-US/US/p/63801) is a built-in 3-axis tilt-compensated electronic compass that was used for measuring latitude, longitude, and altitude of each multi-location study.

Fig 1: Map of Nepal (top) and Sindhuli district (bottom) showing study sites

Management of experiment and trapping of flies

Collection of maggots infested sweet orange fruits (variety: Sindhuli Local) during the peak infestation period was accomplished on the first week of November 2019 at Golanjor-5, Khaniyakharka, Sindhuli. Those fruits were sliced, opened with a knife, and maggots were allowed to exit out from the fruits. Twenty matured larvae were placed in a plastic jar (dimension: 15 cm height and 10 cm perimeter) filled with garden soil (20.7% moisture, loamy soil) up to 12 cm height, replicated 10 times on the 6 November, 2019. Jar opening was closed with muslin piece and rubber band to avoid larval escape from the container. Pupae were examined and counted by gently stirring the soil after two months and placed in the same containers, again fastened with a piece of nylon mesh. Total adult flies emergences along with pupal mortality were recorded. Agile flies made knockdown with ethyl acetate imbibed cotton swab and transferred to microscopic study for morphological identification.

Data collection and statistical analysis

Adult flies in each jar were monitored, counted, and recorded daily at each study site from January to April 2019. At the same time, the digital thermo-hygrometer was used to monitor daily minimum and maximum temperatures for each study site. Microsoft Excel spreadsheet (version 97-2003) was used to derive mean and standard error of mean on percent pupation and adult emergence, and prepared necessary tables and graphs. Student 't-test was used for the mean comparison of days to adult emergence from the pre-pupae (both from sweet orange and lemon) between lower (Sindhulimadi) and higher (Khaniyakharka) elevations.

Results

Phenological parameters of B. minax

Chinese citrus flies have four stages in their life cycle (egg, maggot, pupa, and adult). Its pupation percentage from matured maggots (pre-pupae) was recorded higher in higher elevation, (91.50 ± 1.92) than in lower elevation, (86.50 ± 2.32). Similarly, pre-pupae from infested sweet orange fruits resulted in a higher percentage of pupation (90.25 ± 2.06) than from lemon (87.75 ± 2.30). However, adult fly emergence percentage was similar (>93%) in both altitudes and citrus fruits (Tables 1 and 2).

Table 1: Effects of altitude on the phenological parameters of *B. minax*

Location, Altitude	Pupation % ± SE (n = 200)	Adult emergence % ± SE (n = 200)
Sindhulimadi, 503 masl (lower altitude)	86.50 ± 2.32	93.93 ± 1.44
Khaniyakharka, 1341 masl (higher altitude)	91.50 ± 1.92	93.59 ± 1.14

Table 2: Effects of citrus types on the phenological parameters of *B. minax*

Citrus fruit	Pupation % ± SE (n = 200)	Adult emergence % ± SE (n = 200)
Sweet orange (*Citrus sinensis*)	90.25 ± 2.06	93.42 ± 1.28
Lemon (*Citrus limon*)	87.75 ± 2.30	94.10 ± 1.31

Days to adult emergence

The number of days to adult emergence was significantly different between higher and lower altitudes, (t (38)=28.78, p=<0.001) with the mean difference of 22.45, the higher the altitude, the longer was the duration of adult emergence,

where adult fruit fly emerged out on the 3rd week of April, i.e. it required an average of 165.5 days for the maggots from sweet orange and 164.2 days from lemon, while it completed in 143.5 days for the maggots from sweet orange and 141.3 days from lemon, i.e. adult flies emerged out on the last week of March at lower altitude (Figure 2). At the lower elevation, Sindhulimadi, the highest number (5) of adult flies emerged on the 140th day followed by 4 adult flies on the next day. While it required 162 days for the highest number of adult flies (5) to emerge out at higher elevation Khaniyakharka. The frequency distribution of the days of adult emergence clearly shows the variation at higher and lower elevations (Figure 3).

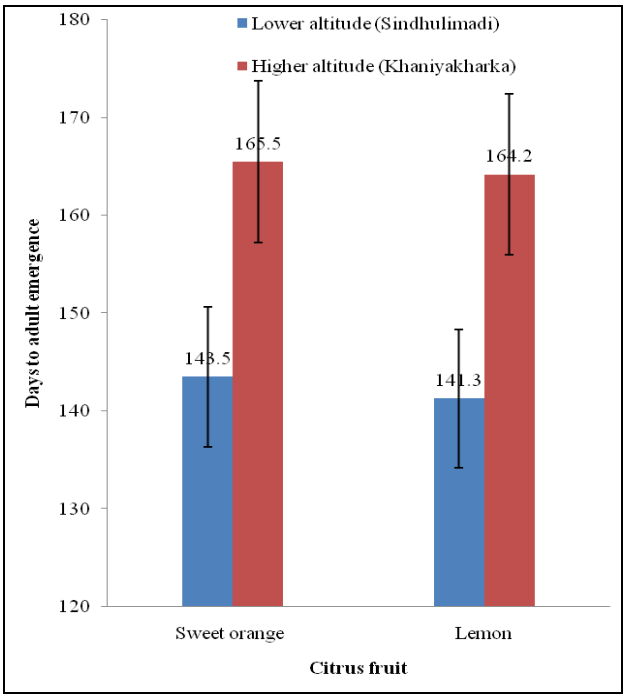

Fig 2: Mean days to adult emergence

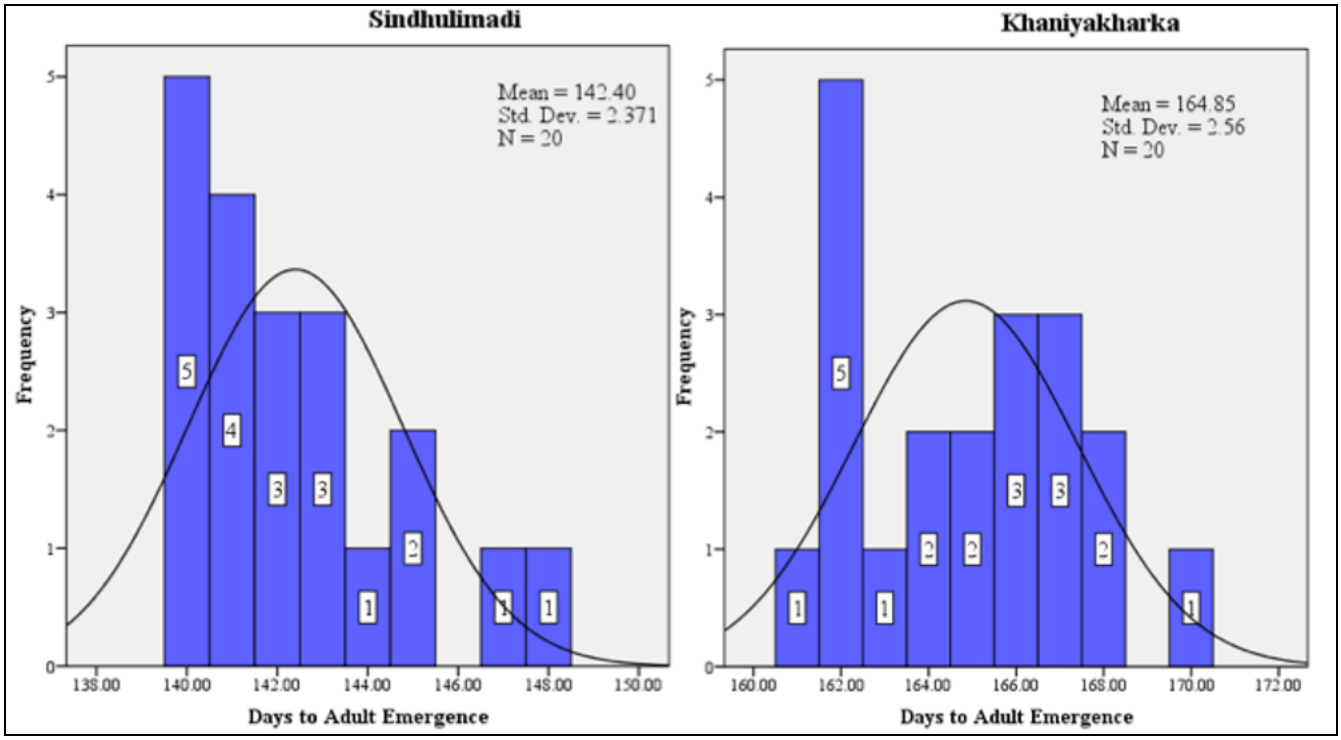

Fig 3: Frequency distribution of the days of adult emergence

Discussion

Phenological parameters of B. minax

The pupation percentage depends on the thermal response of the locality (Yasuda *et al.* 1994) [13]. Hulthen and Clarke, 2006 [14] presented the higher pupation percentage of *Bactrocera tryoni* in loam soil at 75% soil moisture than at either 0% or 100% soil moisture condition. The pupation of fruit fly depends on the temperature, soil type, compaction, and moisture condition of pupation media (Dimou *et al.* 2003) [15]. Thapaliya *et al.* (2020) [16] mentioned 30.06%

pupal mortality of *B. minax* in the Sindhuli district.

Days to adult emergence

This variation to adult emergence from pre-pupae might be due to the temperature condition of the experimental sites, Sindhulimadi at lower elevation and Khaniyahkarka at a higher elevation. Therefore, lower temperature at a higher elevation at Khaniyakharka (17.55 ^{0}C), RH 66.29%; and higher temperature at a lower elevation at Sindhulimadi (18.35 ^{0}C), RH 74.04% during the rearing period influenced the pupal period. The highest numbers of B. minax adult flies were trapped in protein bait based trap during May-June, 2018 at higher altitude (1200-1400 masl) on sweet orange orchards in Sindhuli district (Gautam *et al.* 2020) [17]. Chilling temperature and duration Affect on the pupal developmental period and eclosion. Increasing cold treatment led to generally and considerably rapid adult emergence of fruit fly (Yong *et al.* 2013) [18]. Environmental temperature is one of the major factors when predicting diapause duration of *B. minax* pupae (Zhou *et al.* 2020) [19]. Pupal stage of *B. minax* was the longest and lasted up to 5-7 months depending on the temperature (Wang and Luo, 2018) [11]. Ma *et al.* 2019 [20] reported temperature as the major factor for the days to adult emergence. In western Bhutan, a similar result was published by Dorji *et al*. 2006 [21].

Conclusion

Citrus is an important fruit crop, and the Chinese citrus fly has become one of the devastating pests in the citrus orchards of Nepal. The adult emergence from diapause pupae of Chinese fruit fly varies greatly with the prevailing temperature of the specific location. In lower altitudes, the adult flies emerge earlier than in higher altitudes. This finding clearly shows that the life cycle and behavior of the pest is related to the location and environmental factors, which is crucial in determining the timing in its management strategy.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contribution

DA coordinated in all research activities, data analysis, and write-up of the manuscript; RBT contributed to the research design, reviewed and advised for a write-up; SLJ contributed to the research design and advised for an overall write-up; JJD contributed for the concept of experiment and corrected write-up, YDGC reviewed on the manuscript.

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