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# Survey of the Chinese Citrus Flies *Bactrocera* (*Tetradacus*) *minax* (Enderlein) (Diptera: Tephritidae: Dacinae) Infesting Citrus in Darjeeling and Kalimpong Districts of West Bengal

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**A B S T R A C T**

Darjeeling mandarin (*Citrus reticulata* Blanco) is one of the most popular fruit crops of Darjeeling and Kalimpong districts of West Bengal, India. The cultivation of the crop is significantly affected by the attack of Chinese citrus fly *Bactrocera* (*Tetradacus*) *minax* (Enderlein) (Diptera: Tephritidae). Survey was conducted in the months of November and December of 2017 and 2018 to find out the dimension of the problem along with its distribution, detection of alternative hosts etc. During the survey, 600 samples were collected from 323-1610 meters above sea level (MASL) out of which on an average 50.83% of the fruit samples were found to be infested by the fly. However 10.40-75.18% of the fruits were found to be infected by the pest during harvesting in different orchards. Citrus hosts infested by *B.minax* include Darjeeling mandarin (*Citrus reticulata* Blanco), Sweet orange (*Citrus sinensis* Osbeck), Rough lemon (*Citrus jambhiri* Lush) and kinnow mandarin (*Citrus reticulata* Blanco). Rangpur lime (*Citrus limonia* Osbeck), had been found to be infested by *B. minax* along with *Ptecticus sp* (Diptera: Stratiomyidae) from harvested and fallen fruits. Report on range of hosts infested by *B. minax*, recorded during study was first of its kind from India.

## Introduction

Darjeeling mandarin commonly known as *Citrus reticulata* Blanco, which is one of the important fruits grown in the hilly regions, mainly Darjeeling and Kalimpong districts of West Bengal, India. Insect pests are the major constraint in obtaining good quality fruits and high yield of Darjeeling mandarin (Kumar *et al.,* 2015). Amongst those *Bactrocera* (*Tetradacus*) *minax* (Enderlein) (Diptera: Tephritidae), commonly called as Chinese citrus fly had been recognized as one of the most devastating pests of this fruit (Wang and

Luo 1995; Dong *et al.,* 2014; Liu *et al.,* 2015). It had been reported to be well distributed to a wide range of temperate regions of Asia, including Nepal, Bhutan, China and India (West Bengal and Sikkim) (Fan *et al.,* 1994, Dorji *et al.,* 2006, Drew *et al.,* 2006; Jha *et al.,* 2019). It is univoltine, oligophagous pest and can attack different types of citrus fruits (Zhou *et al.,* 2012). The female fruit flies puncture the peel and lay eggs into the soft and tender fruits (Zhang 2007). Maggots feed inside the developing fruits causing rapid decay, which later become inedible and finally dropped prematurely (Wang *et al.,* 2009). Larvae jump out of the fruits; pupate in the soil which has been found to be longest life stage (Li *et al.,* 2019).Though the pest is very serious for mandarin orange in Darjeeling and Kalimpong districts of West Bengal, non systematic study had been taken up on it. Hence, in the present study, collection of fruits have been done in several localities in Darjeeling and Kalimpong districts of West Bengal to study the occurrence of fruit flies associated with citrus crop, as host.

## Materials and Methods

The present survey work was undertaken during November-December of 2017 and 2018 at Kalimpong and Darjeeling located at northern hilly terrains of the state of West Bengal. Roving surveys were carried out in farmers' fields at different elevations to understand extents of the occurrence and spread of the pest at different altitudes (Fig 9). Random sampling of fruits before harvesting was done to know the percent damage. Initially 19 citrus growing areas were selected for conducting this survey (Fig 10). Total 30 (at least 1-3 locations from each area) mandarin growing orchards were chosen to collect the fly infested samples. The orchards were classified as managed, moderately managed and unmanaged, based on practices of tillage, weed control, application of irrigation, manuring, fertilization, pesticide application and destruction of infested fruits (Table 1). To recover the pupae from infested fruits, collected samples were brought to the laboratory. Then the collected samples were carefully dissected and examined under microscope (Zeiss stemi 2000-C) to get different stages of fruit fly larvae. Infested samples were placed in glass container(30×30×30cm) under laboratory condition. The glass containers were filled with sterile fine sand up to 10 cm height for pupation. The mouths of the containers were tied with muslin cloth until the larvae pupated. The pupae were kept in separate glass container partially filled with moist sand. Emergences of adults were also recorded. The emerging fruit flies were counted and preserved as dry specimen for identification. All the samples were identified at the laboratory, Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur and sample specimens were sent to NBAIR, Bengaluru for confirmation of identity of the particular species. To determine the relations amongst altitudes and types of orchards related to infestation one way analysis of variance had been performed with the helping of SPSS Software Statistics.

## Results and Discussion

In the present survey total collected fruit samples were 600, out of which 305 samples (50.83%) had finally been found to be infested by the fly. However at fruit harvesting season the level of fruit infestation in the orchard had been found to be within 10.40-75.18%. Variation in percentages of infestations could be found in different orchards at different altitudes. At Upper Bong Busti and Tari Gaon Kalimpong only two managed orchards could be found which had lowest (10.40% & 15.27%) fruit fly infestations (Table 4). In moderately managed orchards of Gorubathan

Khasmahal and Sombary Bazar of Kalimpong the levels of infestations were in between 17.44-32.50% respectively. On the other hand unmanaged orchards had showed highest fruit fly infestations having 40.09% and above. The unmanaged orchard at Takdah had highest fruit fly infestation (75.18%) (Table.5). During study the pest could be detected at varied altitudes from 323 to 1610 meters above sea level(MASL) (Fig 9) the infestation of the pest was observed to be highest during first harvesting period which increased significantly with increase of elevation.

The relationship between different altitudes with infestation of fruit fly indicated that maximum infestation was found in mid altitudes sequentially followed by high altitudes and low altitudes respectively (Table 2). Significant increase in fruit fly infestation was found in mid altitudes and those at par with the infestation at high altitudes. Whereas higher altitudes showed a positive significant difference in fruit fly infestation with lower altitudes. Mid altitudes also showed a positive significant difference in fruit fly infestation with lower altitudes (20.95*). However, infestation at lower altitudes showed negative significant difference with both of higher altitudes (-15.73*) and mid altitudes (-20.95*). Similarly the intensity of infestation also varied with the level of management. Maximum infestation was found in unmanaged orchards followed by moderately managed orchards and managed orchards (Table 3). However, the infestation at moderately managed orchard was statistically at par with that of managed orchard. Managed (-27.58*) and moderately managed (-20.45*) orchards showed negative significant differences in fruit fly infestation with unmanaged orchard. Whereas unmanaged orchard showed positive significant differences in fruit fly infestation with managed (27.58*) and moderately managed orchard (20.45*).

The incidence of the adult of *Bactrocera minax* could be recorded in the orchard only when they got sexually matured after ecolsion from the pupae. Egg laying generally took place in mid-June to mid-July. No adult fly could be found in the orchards at end of July. In July, ovipositon spots could be seen on the upper half of fruit surface (Fig 1). *B. minax* preferred laying eggs in young tender fruits with diameters of almost 10-12 mm. Needle like brownish spots of 1-1.5 mm diameter could be observed just under the skin of fruits (Fig 2). In October the maggots became easily visible inside the infested or dropped fruits (Fig 4). The maggots fed on the pulp and developed inside the maturing fruits. The infested fruits gradually became pale yellow or green. The maggots were found to feed inside, rendering the fruits useless and causing them to drop prematurely. When the maggots were matured they left the fruit by making an exit hole (2.80-3.15 mm) (Fig 6), and entered into the soil for pupation (Fig 7).

Premature fruits dropping could be observed in the month of late October to mid-December. This can be considered as most diagnostic symptom at field conditions before the harvesting of the fruits (Fig 3). To take stock of infestations of *B. minax* in mandarin orange and other related crops, be it cultivated or wild, fruits were randomly collected to ensure whether they were containing fruit fly larvae or not.

The pest was abundant in Darjeeling mandarin (*Citrus reticulata* Blanco. local name Suntala), Sweet orange (*Citrus sinensis* Osbeck. local name Mousombi), Rough lemon (*Citrus jambhiri* Lush. local name Naitey Jyamir), Rangpur lime (*Citrus limonia* Osbeck. local name Chayaksey) and also in Kinnow mandarin (*Citrus reticulata* Blanco). The adults emerged from the pupae recovered from the infested fruits were unexceptionally *B. minax*.

**Table.1** Classification of orchards based on different types of practices during the survey

| Application of Practices | Managed Orchard | Moderately Managed orchard | Unmanaged Orchard |
|---|---|---|---|
| **Tillage practices** | Yes | Yes | Infrequently |
| **Weed Control** | Yes | Yes | No |
| **Irrigatioin** | Yes | Yes | Infrequently |
| **Manuring** | Yes | Yes | Infrequently |
| **Fertiliser application** | No | No | No |
| **Destruction of infested fruits** | Yes | No | No |
| **Pesticide application** | No | No | No |
| **Any Other** | No | No | No |

**Table.2** Relationship between different types of altitude with infestation

| Altitude | | Mean Difference | Sig.[a] |
|---|---|---|---|
| **Higher altitude** | Mid altitude | -5.22 | .139 |
| | Lower altitude | 15.73* | .001 |
| **Mid altitude** | Higher altitude | 5.22 | .139 |
| | Lower altitude | 20.95* | .000 |
| **Lower altitude** | Higher altitude | -15.73* | .001 |
| | Mid altitude | -20.95* | .000 |

*The mean difference is significant at the .05 level.

**Table.3** Relationship between different types of orchard with infestation

| Types of orchard | | Mean Difference | Sig.[a] |
|---|---|---|---|
| **Managed** | Moderately managed | -7.13 | .118 |
| | unmanaged | -27.58* | .000 |
| **Moderately managed** | Managed | 7.13 | .118 |
| | unmanaged | -20.45* | .000 |
| **unmanaged** | Managed | 27.58* | .000 |
| | Moderately managed | 20.45* | .000 |

*The mean difference is significant at the .05 level.

**Table.4** Incidence of *Bactrocera minax* in major mandarin growing areas of Kalimpong districts of West Bengal.

| District | Location | Level of management | Latidude and Longitude | Height of the orchards | Percent of infestation | spp |
|---|---|---|---|---|---|---|
| **Kalimpong** | Borbot | Managed | N27.05918 E88.47186 | 1099.2m | 10.40 | *B. minax* |
| | Tarigaon | Managed | N27.05536 E88.47739 | 941m | 15.27 | |
| | | Unmanaged | N27.05542 E88.47753 | 936m | 40.09 | |
| | Bong Busty | Unmanaged | N27.06348 E88.47396 | 1100.7m | 45.56 | |
| | | Unmanaged | N27.06409 E88.47425 | 1093.6m | 42.37 | |
| | | Unmanaged | N27.06415 E88.47438 | 1091.3m | 46.51 | |
| | Mongbol busty | Unmanaged | N27.06331 E88.45874 | 1133m | 74.65 | |
| | Pankhasari Khasmahal | Unmanaged | N27.02672 E88.68463 | 771m | 40.66 | |
| | Gorubathan Khasmahal | Moderately managed | N26.96818 E88.70573 | 535m | 19.52 | |
| | | Moderately managed d | N26.97152 E88.70705 | 566m | 27.45 | |
| | | Moderately managed | N26.9696 E88.70636 | 548m | 23.50 | |
| | | Moderately managed | N26.96919 E88.70602 | 541m | 25.75 | |
| | | Moderately managed | N26.97635 E88.69714 | 414m | 32.50 | |
| | Nim Khasmahal | Unmanaged | N27.02283 E88.67335 | 979m | 62.20 | |
| | Sombary Bazar | Moderately managed | N26.95429 E88.69525 | 323m | 17.44 | |

**Table.5** Incidence of *Bactrocera minax* in major mandarin growing areas of Darjeeling districts of West Bengal

| District | Location | Level of management | Latidude and Longitude | Height of the orchards | Percent of infestation | Spp |
|---|---|---|---|---|---|---|
| **Darjeeling** | Bara Mungwa | Unmanaged | N27.05959 E88.40096 | 883m | 64.50 | *B.minax & Ptecticus sp* |
| | Sitong Khasmahal | Unmanaged | N26.93239 E88.37482 | 907m | 60.25 | |
| | | Unmanaged | N26.93075 E88.37376 | 892m | 64.22 | |
| | Rolak Khasmahal | Unmanaged | N26.94284 E88.40103 | 1242m | 53.65 | |
| | Takling Khasmahal | Unmanaged | N27.04211 E88.40455 | 892m | 70.25 | *B. minax* |
| | Soriang Khasmahal | Unmanaged | N27.03701 E88.40681 | 952m | 69.80 | |
| | Takdah | Unmanaged | N27.06024 E88.40131 | 857m | 75.18 | |
| | Lamahatta | Unmanaged | N27.05209 E88.34089 | 1610m | 42.25 | |
| | School Gaon, Sittong | Unmanaged | N26.93616 E88.37545 | 820m | 48.53 | |
| | Ambootia Tea Garden | Unmanaged | N26.88513 E88.25954 | 988m | 40.28 | |
| | Mirik Khasmahal | Unmanaged | N26.89328 E88.20911 | 1260m | 54.60 | |
| | | Unmanaged | N26.89613 E88.20233 | 1181m | 69.75 | |
| | School Dara 2, Mirik | Unmanaged | N26.89919 E88.19803 | 1293m | 47.38 | |
| | | Unmanaged | N26.89814 E88.19865 | 1315m | 45.25 | |
| | | Unmanaged | N26.89997 E88.19832 | 1263m | 51.30 | |

**Fig.1** Ovipositon spot under the skin of the fruit

**Fig.2** Brownish spot

**Fig.3** Imamature fruits dropping

**Fig.4** Infested fruits

**Fig.5** Third instar larva and exit hole

**Fig.6** Pupa

**Fig.7** Adult female (*B. minax*)

**Fig.8** Adult female (*Ptecticus sp*)

**Fig.9** Infestation of *B. minax* on mandarin orange at different altitudes (meter)

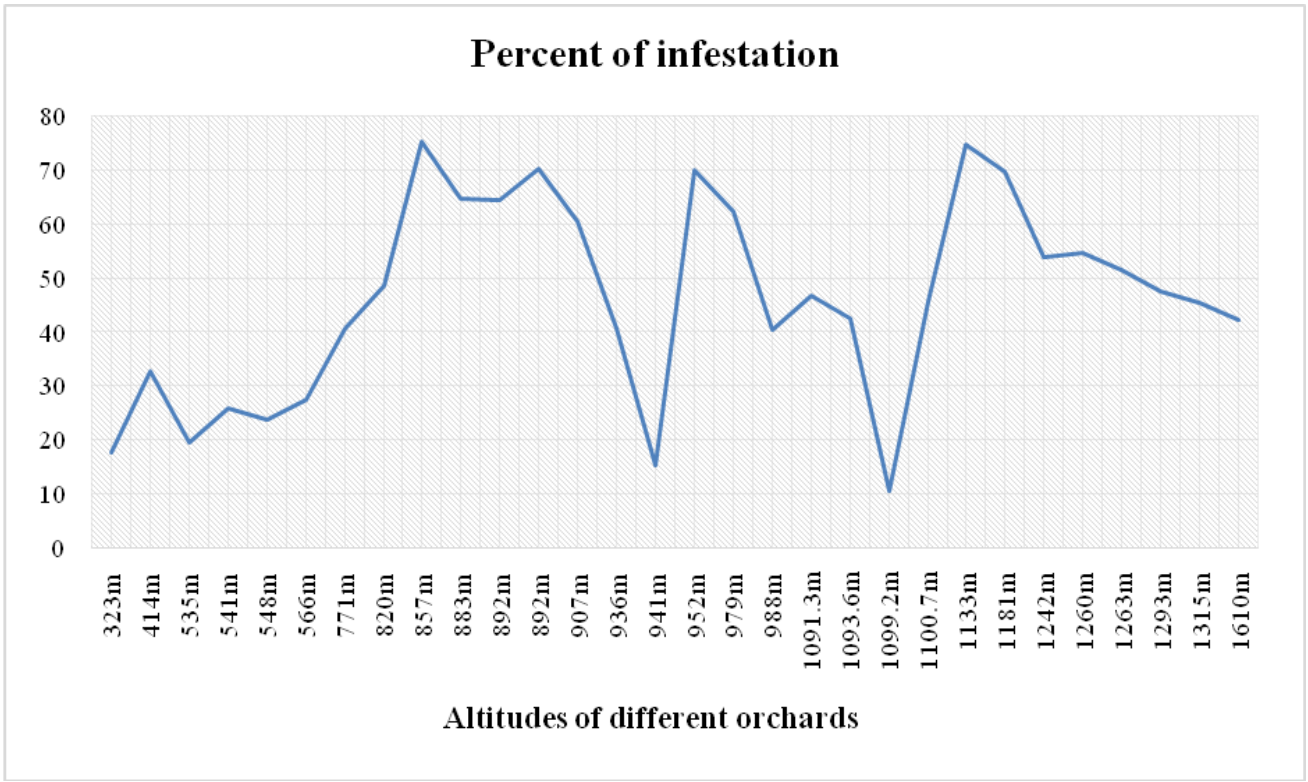
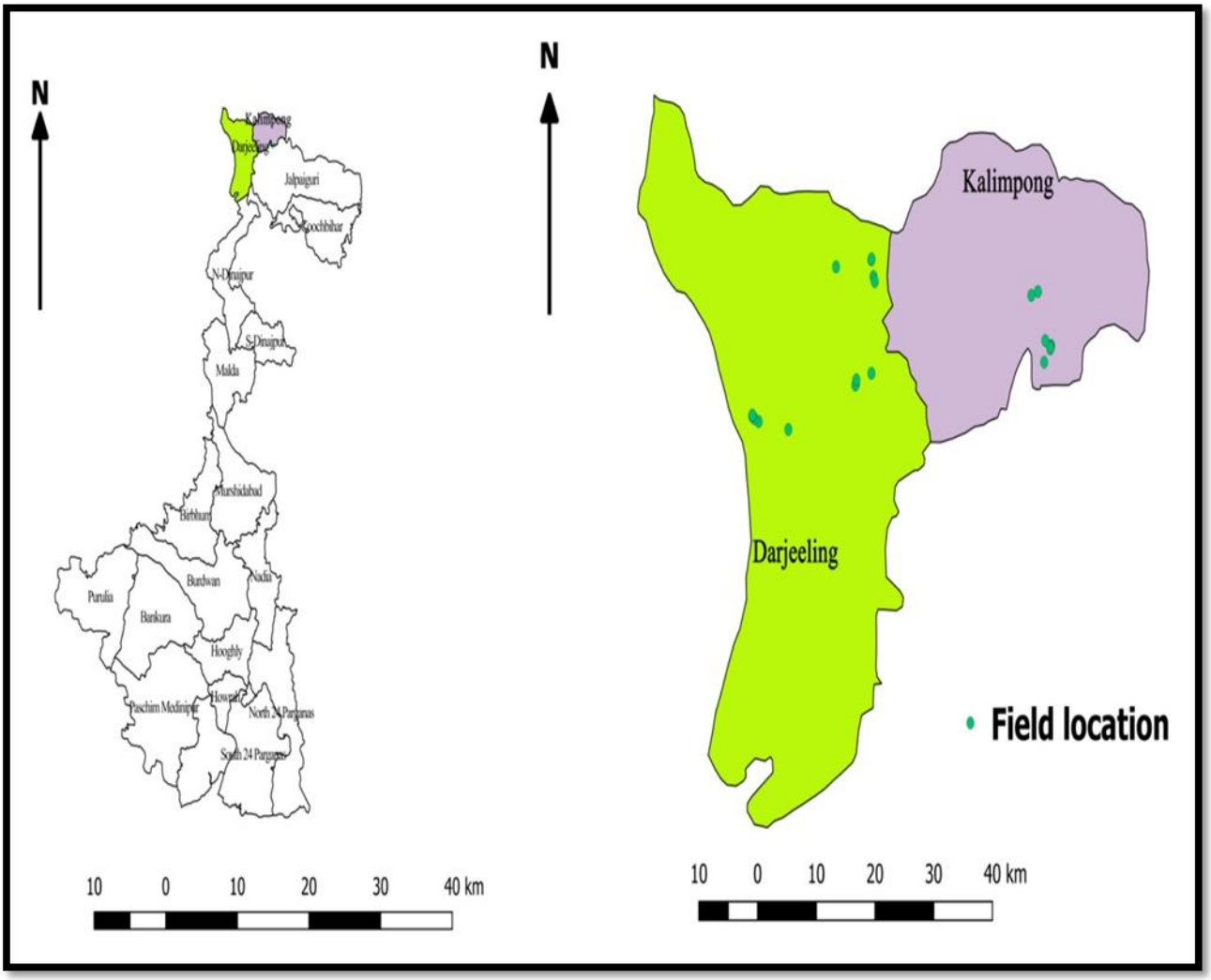


**Fig.10** Survey area of Darjeeling and Kalimpong districts of West Bengal, India




However, *Ptecticus sp* (Diptera: Stratiomyidae) could be recorded only in dropped fruits of rangpur lime in the survey areas.

Adult *B. minax* is clearly different from other species of *Bactrocera* (Fig 8). Total body length; 13-15 mm wing span; 10-11 mm. Face fulvous with, elongate black spot on each furrow. Males are similar to females, however they are smaller. Scutum is reddish-brown in colour, with lateral and medial postsutural yellow vittae. Scutellum yellow with brown basal margin and a pair of apical scutellar setae; postpronotal lobe and notopleuron yellow/fulvous. Wing hyaline with broad costal band overlapping vein $R_{4+5}$, Anatergite and katatergite yellow, haltere fulvous. All femora fulvous with fuscous markings, tibia fulvous with apical fuscous markings, 5 segmented tarsi. The female oviscape is elongated and bottle-shaped. The larvae are apodous, dull white, and have black mouth parts. The third instar was 14-15 mm in length and 2.50-2.80 mm broad. Puparium cylindrical, barrel shaped and 11 segmented, whereas pre pupa was yellowish and mature pupa red-brown in colour. Pupae had an

average length and breadth of 7-8 mm and 3-3.5 mm, respectively.

The survey results were in agreements with van schoubroeck (1999) who reported that the pest infestations were common in orchards of mid and high altitude. *B. minax* had been reported from Southern China, India (including West Bengal and Sikkim), and Bhutan (Drew *et al.,* 2002). Whereas Laskar *et al.,* (2016), reported that both *B. minax* (Enderlein) and *B. dorsalis* could be recovered from mandarin in orchards of Darjeeling district of West Bengal. The present study though confirmed widespread occurrence of fruit fly in mandarin at Darjeeling and Kalimpong districts but fruits were unexceptionally infested by *B. minax* only. Finding of the present study were in corroboration with the earlier works of Guo *et al.,* (2008) and van Schoubroeck, (1999), who reported that female flies laid eggs mainly in the young fruits, after the first mandarin fruit reaching 11mm diameter, the first stage susceptible to fruit fly oviposition. Guo *et al.,* (2008) reported that the fly infested fruits skin turned yellow with some red spots, the fruits later fell down. However Zhang *et al.,* (2007) reported that the oviposition punctures by the fly were noticeable. The obtained result on extent of damage was in conformity with Wang *et al.,* (1995); Nie *et al.,* (1999) and Dorji *et al.,* (2006) who reported that the Chinese citrus fly, *B. minax,* an oligophagous pest causing more than 50% yield loss and occasionally it could be upto 100%. Earlier Kumar *et al.,* (2015) identified the fruit fly as *Bactrocera dorsalis* and Laskar *et al.,* (2016) reported a mixed population of *B. minax and B. dorsalis* recovering from mandarin orange of Darjeeling district, West Bengal. The finding of the present study did not confirm it. No *B. dorsalis* had been found to attack *Citrus reticulata* during the present experiment. Dorji *et al.,* (2006) who conducted such study in Bhutan, adjoining to the present area of experimentation, confirmed the fly species infesting mandarin orange both at commercially grown area and wild was *B. minax*. Acharya *et al.,* (2019) from Nepal also reported that the only tephritid fly infesting mandarin orange was *B. minax*. So far as ranges of hosts are concerned Allowed *et al.,* (1999) also reported infestation of *B. minax* on various wild and cultivated species of citrus in South East Asia. Lin *et al.,*(2011) reported that *B. minax* preferred laying eggs on navel oranges and other sweet orange cultivars. Yang *et al.,*(1994) also reported from Hubei province of China that *B. minax* was one of the major fruit fly pests on citrus trees.

*B. minax,* an oligophagous, univoltine pest of *citrus reticulata* had been found to cause serious damage to mandarin fruits in the unmanaged orchards specially located at 323 m to1610 m altitude. The detection of infestation of the pest also became difficult due to confusing symptoms expressed externally. The adults, as typical of tephritid, went for sheltering in non-host plants which had made it further difficult to locate its presence.

The gravid females, started ovipositing in mid June-July which coincided with rainy season in the region, detection of *B. minax* in orchards at that period became further difficult. Through the present study some location specific critical information from Kalimpong and Darjeeling had been generated on the life strategies of *B.minax* which will be of immense help in managing the fly. This study helped to document the diversity of *B. minax* of the Northern part of West Bengal and has created a base data of *B. minax* diversity that can attract and support future extensive studies in the area.

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