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Mandarin Yield Loss and Pupal Density Assessment of Chinese Fruit Fly at Citrus Pockets of Gandaki Province, Nepal

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Abstract

Owing to the superior quality and taste, Nepalese citriculture, especially the mandarin, is famous both at local and global market. However, recent invasion of Chinese fruit fly (CFF) *Bactrocera minax* (Enderlein) has posed a serious export threat of the mandarin. A semi-structured questioning with commercial citrus farmers of Gandaki Province to unveil the CFF-related issues and a field level assessment of the CFF pupal density to predict the future damage potential of the pests were carried out. The study showed that the mandarin growers were facing sever CFF-infestation since 3-4 years. They reported that the fruit drops started in July-August and peaked in September of the season leading into an average yield loss of 16.63±1.68% in Gandaki Province, the highest (20.94±4.34%) suffered by the farmers from Syangja. Though 64% of the respondents were adopting collection and disposing of the fallen infested mandarin fruits which led into a significant decrease (78.95±5.69% in 2021 and 72.48±3.17% in 2022) in CFF pupal population, there were plenty of larvae gone for pupation which could be sufficient to infest the next-season mandarin. Moreover, ~ 22% of the respondents were not caring the fallen fruits which could be the major next-season source of the CFF adults. So, an area-wide control measure focusing on orchard sanitation, chemical sprays and baiting could be the immediate action for the control over CFF. However, investigation on the pest phenology in Nepalese context, search of wild hosts of the CFF and immediate technical backstopping to the citrus communities are imperative.

Keywords: *Bactrocera minax*, fruit drop, mandarin, questionnaire, sampling

Introduction:

Chinese Fruit Fly (CFF), *Bactrocera minax* (Enderlein) (Diptera: Tephritidae), has now become the serious threat to the Nepalese citriculture. The global citrus industry is also seriously threatened by the pest (Dong et al., 2014 a, b). In severe situations, the pest can inflict a total seasonal failure of the citrus crops (Allwood et al., 1999). Univoltinism present in the CFF is unique among the sub-family Dacinae tephritids (Fletcher, 1989). However, Dong et al. (2013) reported to be multivoltine oligophagous species of the most preferred host sweet orange (Xia et al., 2018). The oligophagous pest, strictly confined to the citrus species (Allwood et al., 1999), has been recorded from China, expanding through West Bengal and Sikim of India, through the Himalayan Kingdom Bhutan (Drew et al., 2006; Wang & Luyi, 1995) and now to the Eastern mid-hills of Nepal (Drew et al., 2006). Moderate temperature with lower humidity, as found in mid-hilly regions from March April to June-July, are some conducive environment for the exponential increase of the fly population (Xia et al., 2018).

Mid hilly belt of Nepal is renowned for quality exportable mandarin. However, the major limiting factors for the production of exportable standard crops are insect pests. Amongst the pest insects responsible for yield loss of mandarin, CFF is considered the major one (Thapaliya et al., 2020). CFF, a native of China, is reported in Bhutan, India, Nepal and Viet Nam (Dorji et al., 2006; Drew et al., 2006). Since 2014, the CFF has travelled from China through Bhutan and western hilly parts of India to eastern Nepal infesting sweet oranges of eastern mid-hills (Drew et al., 2006; Sharma & Dahal, 2020). Its territory is assumed to extend from east to west of Nepal and the resulting fruit damage has exerted a huge economic losses to the growers. Adhikari et al. (2020) reported around 15% eastern hilly mandarin yield losses to the pest. Sharma et al. (2015) reports the possibility of causing up to 97% yield loss of

Eastern hilly sweet orange by the time of harvesting. However, it is limited to 35-75% at the mid and high altitude orchards in Bhutanese and Chinese contexts (Dorji et al., 2006; Xia et al., 2018). Adhikari et al. (2018) in another report explains around 30% fruit damage by this exotic pest.

CFF is an oligophagous pest (Xia et al., 2018) and the host range has been recorded in citron (*Citrus medica* L.), lemon (*Citrus limon* L.), meiwa kumquat (*Fortunella crassifolia* Swingle), pummel (*Citrus maxima* Burm.), sour orange (*Citrus aurantium* L.), sweet orange (*Citrus sinensis* L.), tangerine (*Citrus reticulata* Blanco), navel orange (*Citrus sinensis* Osb. var. brasiliensis Tanaka), grapefruit (*Citrus paradisi* Macfad) and trifoliate orange (*Poncirus trifoliata* L.) (Nath, 1972; Chao & Ming, 1986; Liu et al., 2014). Among these, the preferred citrus host plant is sweet orange (Liu et al., 2014). Female CFFs start to oviposit eggs into small unripe fruit from mid-June to mid-July (Wang & Luo, 1995; Dorji et al., 2006) of the season leaving the oviposition point covered with waxy oviposition marking substance which transform transparent to translucent and to yellowish gradually within 1-3 days, bulges out and cracks (Wang & Zhang, 1993). But in Nepal, female oviposit during March to July (Adhikari et al., 2020). Usually two months is required for the eggs to hatch which is much longer than other species in the *Bactrocera* genus. The larvae go through three larval instars and the larval stage lasts until the end of October, when fruit drop usually peaks in the Nepalese condition, a month earlier to the normal fruit ripening season of the year. Now, its damage has been reported also from western Nepal like Syangja, Gulmi, Lamjung districts (Sharma et al., 2015).

Informally, farmers and technicians were reporting the incidence of the fly since a couple of years back. After a thorough survey by the DoAR-Gandaki (2021) in 2020, the infested mandarin fruits were found harboring the larvae of CFF which developed into CFF adults at the insectarium of DoAR-Gandaki, Lumle. This led to an initiation of the management strategies via Agriculture Knowledge Centers of the respective districts and the Prime Minister Agriculture Mechanization Project (PMAMP). However, the farmers repeatedly reported to incurring losses to CFF. This led to an initiation of this study. We developed a semi-structured questionnaire to unveil some important facts; start of the fruit drop season due to the pests, losses incurred to the pest, status of the management strategies adopted by the farmers and to find the CFF pupal density both at the orchards following some management practices and at those not adopting any control measures.

Materials and Methods:

It includes the purposive survey methods and pupal density assessment from the infested orchards.

Questionnaire survey

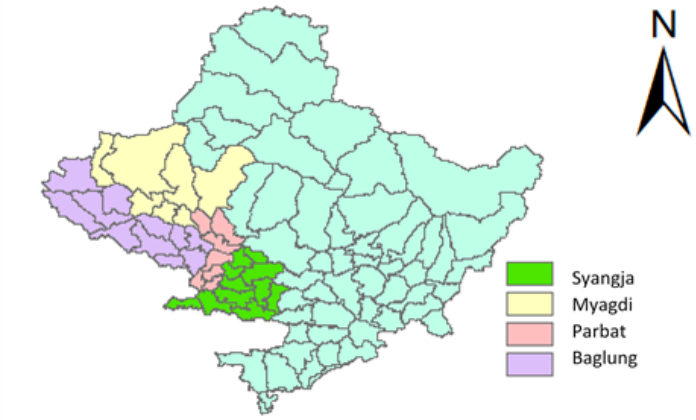

Figure 1. *Districts of Gandaki Province, Nepal selected for interviewing citrus farmers related to CFF issues*

The respondents for the questionnaire survey were purposively selected from four districts of Gandaki Province; Baglung, Myagdi, Parbat and Syanja (Fig. 1). Two citrus pockets from Baglung - Sarkuwa and Damek; two pockets of Myagdi - Bhorleni and Virjula; different locations from Baskharka, Parbat; Bajarmare, Sirane, Tallo Gaun, Upallo Gaun, Okhale and Danda; 3 pockets from Syangja - Dyangdi, Thapathok and Shimle were selected. Fifty commercial mandarin farmers were selected as listed in Table 1.

Besides the educational status and some other basic information, the respondents were inquired for some issues related to CFF; years since the respondents were facing citrus fruit drops, years of commencement of CFF damage, citrus species those are attacked by the CFF, stage of the citrus when the CFF damage start to be notified, nature of damage, months of fruit fall start and the maximum fruit drop month, amount of the citrus losses to the pest, source of knowledge regarding the CFF, management practices adopted and change in the amount of fruit damage by the pest and so on.

Table 1. Number of respondent citrus farmers from four districts of Gandaki Province.

Districts	Municipality	Citrus pockets	No. respondents	Geographical position
Baglung	Jaimini	Sarkuwa, Damek	10	1070 - 1392 m asl 28°09'51" - 28°10'38" N 83°35'44" - 83°36'03" E
Myagdi	Mangala	Bhorleni, Bhirjula	10	1360 - 1620 m asl 28°22'28" - 28°24'13" N 83°27'21" - 83°27'39" E
Parbat	Jaljala	Bajarmare, Sirane, Tallo Goun, Upallo Goun, Okhale, Danda Goun	15	1270 - 1560 m asl 28°22'30" - 28°22'47" N 83°34'60" - 83°35'39" E
Syangja	Putalibazar	Dyangdi, Thapathok, Shimle	15	850 - 1140 m asl 28°04'13" - 28°06'42" N 83°47'29" - 83°54'16" E

Assessing CFF pupal density

The pupal density was assessed from two districts; Parbat and Myagdi in 2021 and from three districts; Baglung, Myagdi and Parbat in 2022. In the field, two types of orchards were purposively selected; the orchards in which the fallen citrus fruits were collected and disposed and those in which the fallen fruits were kept unmanaged. One m^2 (1m × 1m) was marked and dug with a sickle hoe to a depth of a feet where CFF pupae were found at the dry-wet soil interface. The CFF pupae collected were brought into the insectarium of DoAR-Gandaki, Lumle.

Confirming the CFF population

To confirm whether the collected pupae were CFF, they were reared under laboratory condition of DoAR-Gandaki, Lumle. Pupae from every orchards were collected and placed into the vials with holes at the bottom for aeration. After collection of pupae, the vials were first filled with moist soil where the pupae were introduced and again filled gently with the moist soil from the top. The vials with pupae were watered ad lib until they emerged as adults. The emerged adults were compared to those with the authentic literatures (Plant Health Australia, 2022) for the confirmation as CFF.

Data analyses

The data collected through survey questionnaires were analyzed and pictured with simple excel software. The CFF pupal population collected from different orchards were compared with *T*-statistic (R studio, version 4.2.0).

Results:

Fruit drop: years of appearance of the symptom

All the citrus growers were observing the fruit drop symptoms and majority (30%) of them were observing this since last three years. Another 22% of the respondents observing it since last 4 years, 18% respondents since 5 years and 2% of the respondents were experiencing the fruit drops since more than 10 years (Fig. 2).

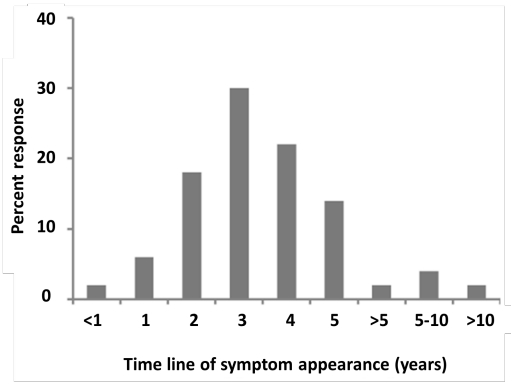

Figure 2. *Years of the symptoms of citrus fruit drops the respondents are suffering from*

Factors causing citrus fruit drops

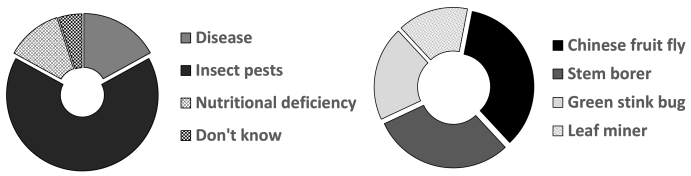

Figure 3. *Respondent's perception on factors of citrus fruit drops (Left) and the major insect pests that cause the fruit drops (Right)*

Looking at the Fig. 3 (Left), it could be easily assumed that the responding citrus growers were suffering from fruit drops mainly due to the insect pest problems (66%) followed by disease (17%) and nutritional deficiencies (12%). A portion of the responding population (5%) were unaware about the causes of the fruit drop. Fig. 3 (Right) unveils the fact that the major insect pest problems the citrus growers of Gandaki Province were facing was the CFF (35%) followed by borers (30%), green stink bugs (20%) and leaf miner (15%). This showed that the major insect pest during the survey time was the well-known CFF.

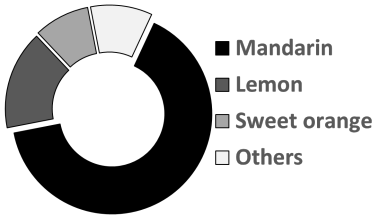

Figure 4. *Respondent's view on the major citrus crops attacked by the Chinese fruit fly*

Majority (65%) of the respondents were facing CFF on mandarin followed by lemon (16%) and sweet orange (9%). A few percentiles (10%) reported the CFF on lime, pomelo and others (Fig. 4).

Time of a crop season the respondents notice the CFF and its nature of damage

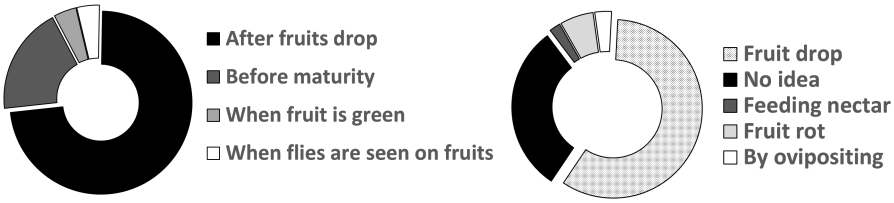

Figure 5. *(Left) When the respondents observed the Chinese fruit fly infestation? And (Right) what damage symptoms they noticed?*

The respondent citrus growers observed CFF infestation on mandarin at different fruit stages (Fig. 5 Left). Majority (73%) came to know the infestation only after the fruits drop. A few percentiles (19%) could see the infestation before fruit maturity, even less respondents (4%) knew the infestation when fruits were green and some (4%) could see the CFF at oviposition time. This showed that the majority of the respondents were unaware about the initiation of the CFF infestation in the orchard. Among the respondents, 58% could see the symptoms of fruit drops (Fig. 5 Right) and a major proportion (30%) had no idea about the CFF's damage symptoms. Some (6%) reported fruits rot and some reported the damage by feeding nectar (2%) and by ovipositing eggs (3%).

Fruit drop months of the mandarin season

Fig. 6 demonstrates the mandarin farmer's response on the start of fruit drops due to CFF and the highest fruit drop months. Accordingly, the fruit drop (16%) started in July, peaked (50%) in August and lowered in September (20%), October (4%), November (6%) and December (2%) of the mandarin fruit season. Similarly, the highest fruit drop (48%) month was reported to be September, followed by November (16%), August (14%) and October (14%). Fruit drop was reported even in December (6%) and Janua ry (2%) of the season.

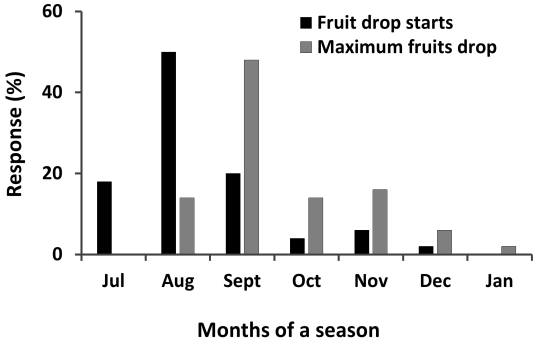

Figure 6. *Fruit drop starting month and maximum fruit drops months of the season*

Mandarin yield losses to the CFF

Percent mandarin yield losses to CFF is presented in Fig. 7. Highest (mean ± SE %) losses (20.94±4.34) was recorded in Syangja district followed by Baglung (18.95±2.20), Myagdi (17.28±4.11) and Parbat (11.75±2.57). The provincial mandarin yield losses to the pest was recorded 16.63±1.68%.

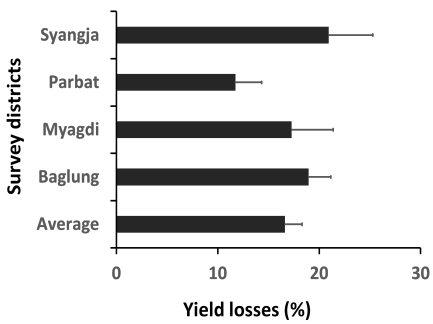

Figure 7. *Mandarin yield losses (%) to the Chinese fruit fly by the respondent growers of citrus producing districts of Gandaki Province.*

Source of knowledge on fruit fly, adopted management practices and the disposing sites of the fallen fruits

More than half of the respondents (64%) knew about the CFF through self-visualization, to the 14% of the respondents, agriculture technicians from municipal offices assisted in identifying the fly, 8% of the respondents came to know through neighbors and 2% respondents through Radio and TV (Fig. 8 Left).

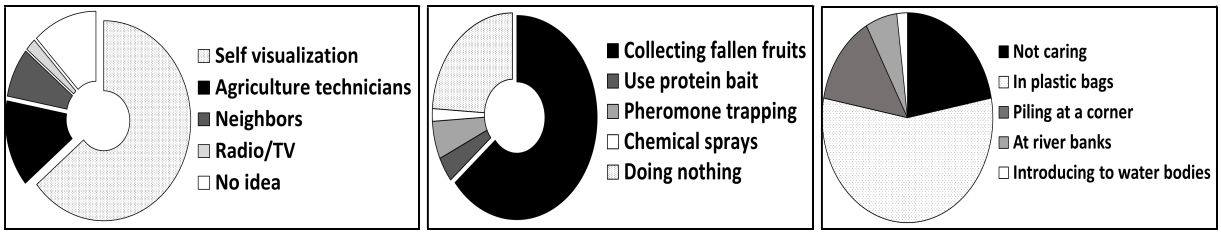

Figure 8. *Respondent's sources of knowledge on fruit fly (Left), management practices adopted (Center) and the sites of disposal of the collected fallen mandarin fruits (Right)*

About 76% respondents were adopting the pest management practices of which 64% practiced to collect dropped fruits from the field, 6% were using pheromone traps, 4% using protein bait and 2% wer e spraying chemicals for the management of the fly (Fig. 8 Center). More than half of the respondents (56%) were found to dispose the dropped fruits in plastic bags, 22% of the respondents left the dropped fruits uncared, 14% were piling at a corner of the terraces, 6% were found to dispose at the river or stream banks and 2% of the respondents answered to introduce the collected fallen fruits into the water bodies (Fig. 8 Right).

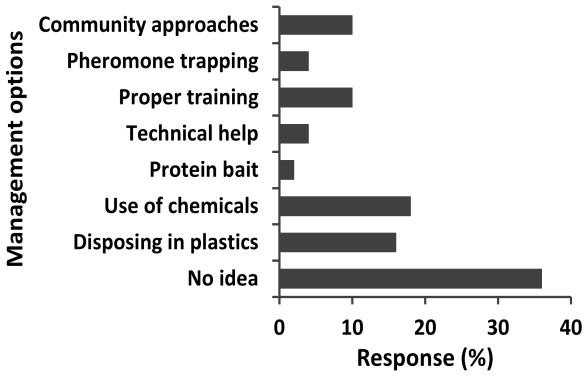

Figure 10. *Respondents' perception on the future management strategies against Chinese fruit fly.*

Years of start of fruit fly management and level of incidence at present

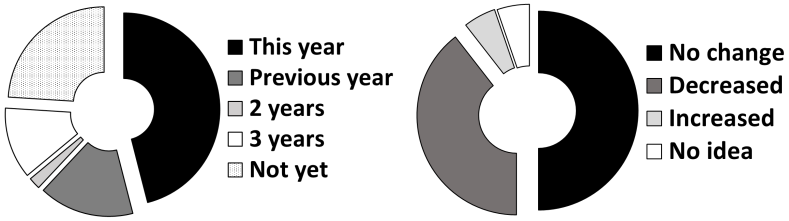

Figure 9. *Years of start of management of Chinese fruit fly by the respondents (Left) and the current trend of fruit fly infestation (Right)*

Fig. 9 (Left) shows the timeline since when the citrus growers of the Gandaki Province were adopting the CFF management practices. Almost half of the respondents (46%) responded adopting the CFF management practices from this year, 16% of the growers since last years, 2% since 2 years and 12% respondents adopting since 3 years. A big proportion of the respondents (24%) was not adopting any control measures against CFF. As of Fig. 9 (Right), 50% of the mandarin growers found no change on CFF incidence after adopting the management practices, 39.48% perceiving decrease in the CFF incidence. A small proportion of the respondents (4%) reported the increase in the CFF incidence even after adopting the management practices and same of the proportion had no idea on the level of incidence.

Possible future interventions to keep the population down

Amongst all, majority (36%) of the respondent mandarin growers didn't see the possibility of future interventions against CFF. Second largest respondent's population (18%) were searching for the possibility of chemical control. Only 16% assumed that the collecting and disposing of the fallen fruits could reduce the CFF infestation. Protein

bait (2%), pheromone trapping (4%), technical help (4%) and technical trainings (10%) were also believed to be the alternatives.

Pupal density of CFF

The hand picking and disposing of the fallen fruits led into the significant (year 2021: $T_{12} = 6.38$, $P < 0.0001$; year 2022: $T_8 = 5.39$, $P < 0.001$) decrease in the pupal density (by 78.95 ± 5.69% in 2021 and by 72.48 ± 3.17 % in 2022) (Fig. 11).

Identification of CFF

The photograph of a female adult specimen emerged from pupae reared at insectarium of DoAR-Gandaki, Lumle is shown in Fig. 12. The morphological features as described by Plant Health Australia (2022) and CABI (2020) were very identical to the emerged adults. This led to a confirmation that the collected pupae and the fly infesting mandarin fruits were Chinese fruit fly.

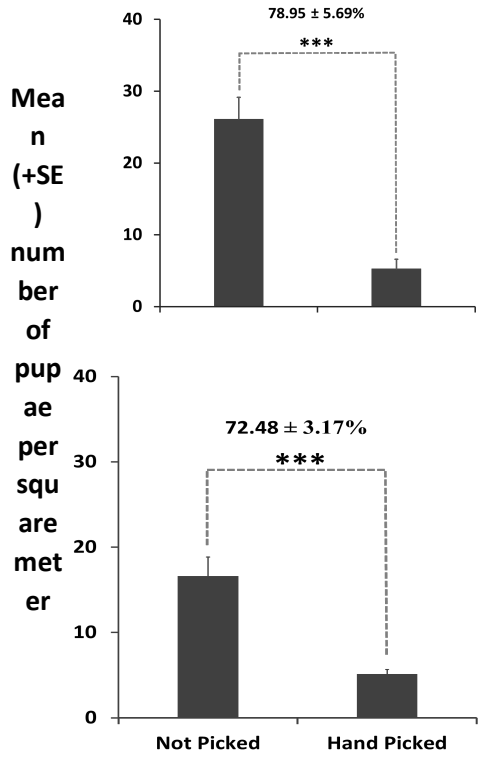

Figure 11. *Number of CFF pupae in 2021 (top) and 2022 (bottom) in a square meter of area and a feet depth from hand-picked of fallen mandarin and not-picked orchards of Gandaki Province.*

Discussion

Though there are not documents explaining the time line of invasion of the CFF to Gandaki Province, our study unveils the fact that the farmers were suffering its damage since more than a decade. However, the level of damage and the suffering citrus growers were a few, in this study only around 2% of the respondents. The CFF hiked to a major pest since a couple of years back when DoAR-Gandaki, Lumle immediately carried out an action research during 2020 (DoAR-Gandaki, 2021) to identify the pest and assess damage level and declared the pest was the CFF (Fig. 12). Since the major citrus of Gandaki Province is mandarin, the damage incurred to the pest is also higher (65% of the respondents) for mandarin as ascribed by this study. A study by National Citrus Research Program (NCRP), Dhankuta confirmed the incidence of CFF since 2006 in Eastern Nepal, especially on the sweet orange (NCRP, 2006). Looking at the time line of the damaging level incidence of the CFF at Eastern and Western Nepal, it could be assumed that the CFF is invading east - west and reaching the citrus belt of Nepal in the nearest future.

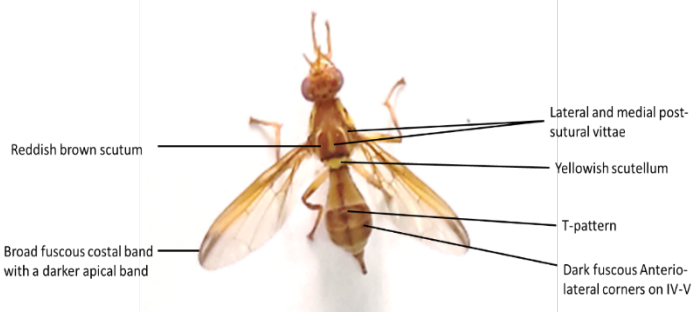

Figure 12. *A Female adult of Chinese fruit fly emerged at the insectarium of DoAR-Gandaki, Lumle and its identical characteristics (Photo by Dr. K Chiluwal)*

The major factor behind its hike during last few years, according to this study was the respondent's lack of know-how about the pest. Majority of the farmers (73% in this study) could realize the incidence only after fruit fall and so 53% of the respondents assumed fruit drop as the main symptom of damage of the pest. Farmers could realize the incidence only after the start of fruit drops during July which hiked during August and continued until December of the fruit season. On the other hand, the maximum fruit drop month in Gandkai Province was September, earlier to the actual fruit ripening season leading into a huge loss in the part of growers. The loss in this study was highest in

Syangja (20.94%) and the Province average was 16.63% which was lower as compared to up to 100% loss in sweet orange as reported by Adhikari et al. (2020). About 50-60% loss was reported by NCRP (2006) in eastern hill districts; Dhankuta, Bhojpur and Khotang. Kingdom of Bhutan, a similar topographies to Nepal, also facing a yearly average 35-75% mandarin losses to the CFF (van Schoubroeck, 1999). Similar level of damages were also reported by Dorji et al. (2006) and Xia et al. (2018), respectively in Bhutan Kingdom and China. In western Bengal, its infestation was about 50.83% (Pashi et al., 2021). The lower mandarin losses to the CFF in Gandaki Province was mainly due to the management interventions adopted by the Agriculture Knowledge Centers (AKCs) and Prime Minister Agricultural Mechanization Project (PMAMP) after an alert issued by the DoAR-Gandaki, Lumle.

Though the yield loss was lower compared to other places and countries, the technical interventions to the citrus growers of Gandaki Province was still lower as proven by the higher degree of dependency of CFF identification on the growers themselves and 64% of the management practitioners adopted collecting and disposing off the fallen fruits of which only 56% were composting the infested fallen fruits in poly bags. This figure was in line with Gautam et al. (2020) where 52.6% of farmers collected the dropped fruits in plastic bags. Surprisingly, 22% of the respondents followed no management practices which could be the sources of CFF for the next season mandarin crops. In between, majority (46%) of the citrus growers were practicing the CFF management practices this year and some since the last two (16%)-three (12%) years. Though majority of the respondents (50% exact) experienced no change in the CFF infestation after adopting some physical and bait methods of pest control, it still remains to lower down the population of CFF through proper technical interventions. So, the respondent growers in majority (36%) had no idea on how to lower down the pest damage in coming seasons. Yet, another big proportion of the respondents (18%) could assume chemical sprays as the best options and only 14% were hoping to follow the disposing of the infested fallen fruits. Since, there were big population (24%) not caring the infested fallen fruits, the pupal population under the plant canopy in such orchards were found significantly higher. The study showed that the collecting and disposing of the fallen fruits significantly reduced the pupal population as compared to the not-cared ones. However, there were a few populations of CFF pupae sufficient to infest the next season crop. Probably, this was a reason why the farmers were facing pest burden even after they were following the management practices. This was supported by a research of Pashi et al. (2021) where they reported only 10.40 and 15.27% infestation in managed citrus orchard as compared to 40.09% infestation in unmanaged orchards of West Bengal. On and above these, the citrus growers had tendency to wait for many days to collect large numbers of fallen fruits until when the larvae inside the fruits might have migrated to soil for pupation. In an area-wide management program as illustrated by Adhikari et al. (2020), Sharma and Dahal (2020) and Xia et al. (2018), the major sanitary practice could be the immediate collection of fallen fruits and subsequent treatments of the produce to meet the phytosanitary obligations.

Conclusion

Since a couple of years, Gandaki Province citrus growers are facing a big challenge of CFF. Though some management practices are started up, they seemed to be insufficient mainly because of a couple of reasons and so the current Provincial average loss of mandarin to the pest is 16.63±1.68%. A big proportion of the citrus growers is not adopting area-wide control of the fly. It comes in the part of agricultural - extortionists to help them understand the importance of area-wide control. The growers also has to know the season of baiting the CFF since their number reaches maximum during earlier than they experience the damage. In Bhutan and China, its population peaks during June-July (Van Schoubro, 1999; Dori et al., 2006; Wu et al., 2008). However, in Nepalese condition, it still remains to be proved through further studies. Gautam et al. (2020) suggested to use protein baits before June as the population starts to decline hereafter.

On the other hand, our citrus growers are using irrelevant pheromone lures of other *Bactrocera* species as they are not responsive to any of the chemical lures; either methyl eugenol or cue lure (Bateman, 1982). Acharya & Adhikari (2019) suggest to use greater fruit fly baits for the are-wide control practice as the bait was much attractive compared to other baits. At the midst, it would be useful to test the attractiveness of color traps impregnated with the baits as they were found to be attractive to orange or green + yellow mixtures (Drew et al., 2006) and other attractants as proven by Zhou et al. (2012) the higher attractiveness of CFF to enzymatical-hydrolyzed beer yeast as

liquid bait compared to other four attracants (GF-120 fruit fly bait, sugar-vinegar-wine mixture, torula yeast and Jufeng attractant).

Even though some farmers were adopting the management practices, they were not able to manage the CFF population to the minimum. This was due to delay in collecting the dropped fruits which led to the immediate migration of pre-pupal larvae to the soil for pupation. So, promptness in collecting and disposing the fallen and infested fruits is crucial to check its next season population. Finally, area-wide control program with the combined use of some techniques; promptly disposing the fallen infested fruits, protein baiting before adult population peaks and some chemical management practices may block the generation build-up of the CFF. However, some important issues like identifying host crops other than the mandarin, sweet orange and lemon and community level technical interventions are imperative for the effective management of the pest and it would greatly help if the phenological studies of the pest were carried out since the population would vary with geographies as reported by Hong et al. (2013) using a one mitochondrial DNA gene fragment (*nad4*) method and demonstrated the high level of genetic diversity among the CFF individuals collected from 18 localities.

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Declaration of conflict of interest and ethical approval:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contribution:

K. Chiluwal designed the study and managed the fund, carried out the study, analyzed the data and drafted the manuscript; R. Ghimire, S. Magar, S. Ghimire and L. Shah carried out the field experiments. All authors finalized the manuscript and accepted to submit for scientific publication.

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