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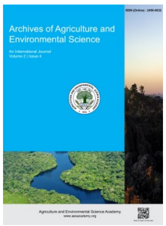


ORIGINAL RESEARCH ARTICLE

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# Present status and future prospects to safeguard Nepali citrus industry against Chinese citrus fly (*Bactrocera minax* Enderlein)

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**ABSTRACT**

Unlike other Tephrid flies, the Chinese citrus fly (*Bactrocera minax* Enderlein) is univoltine and oligophagous species strictly restricted to citrus fruits. It has been a serious threat to the citrus industry in China, Bhutan, India and Nepal causing up to 100% of fruit drop before the harvest. Citrus groves, especially tight-skinned cultivars, sweet orange (*Citrus sinensis* L. Osbeck) in mid-hill districts like Ramechhap, Sindhuli, Dolakha, Kavre, Syangja, Gulmi, etc. have been threatened while in some pockets, lemon, acid lime, and mandarin have vanished due to the Chinese citrus fly (CCF). The driver behind the spread of this invasive pest seems to be poor research works on the phenology of the pest, ill-equipped management practices, flying nature of adult fly and easy movement of infested fruits. Therefore, with reviewing published data, this study aimed to figure out the most appropriate management technology for curbing the CCF and make comprehensive material for safeguarding the citrus industry in the future. Since Area-Wide Integrated Pest Management (AW-IPM) or Area-Wide Control Program (AWCP) was found to be an effective tool to control the CCF, individual practices are crucial to incorporate. Monitoring the pest with the lure of protein hydrolase (PH) and subsequently killing adults with attractive protein baits of 25% hydrolyzed protein + insecticide as lethal dinner is mentioned exceptionally better. In AWCP domestic practice: orchard sanitation is not so effective if the orchards are sloppy while shallow tillage adds less to the natural enemy mechanism of CCF pupae in the soil. Equally, we conclude that Sterile Insect Technology (SIT) is not so economical and the boons of natural enemies, parasitoid and entomo-pathogens against CCF, is yet to be exploited.

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## INTRODUCTION

The Chinese citrus fly (*Bactrocera minax* Enderlein) is one of the most destructive insect pests of the citrus industry in Nepal, China, Bhutan and North Western Himalayan range of India (Chauhan *et al.*, 2019). In severe cases, the loss due to this invasive fly is almost 100% before the end of the harvest season. In the past few years, the fly has been prevailing in most of the tight skinned citrus orchards like sweet orange, pumelo, lemon and lime and comparatively less in loose skinned citrus fruit like mandarin (Adhikari *et al.*, 2019). The CCF is believed to be originated in Northeastern China and made its way to eastern Nepal via Bhutan and Western India (Sikkim). The pest has been reported from even western Nepal like Syangja, Gulmi, Lamjung districts (Sharma *et al.*, 2015). Though early detection in the 1980s, the species was only recognized later in 2007 (Adhikari *et al.*, 2019; Chauhan *et al.*, 2019). Due to misidentification, the fly was taken as *Bactrocera dorsalis* and all the previous works went in vain (NCRP, 2014). It is one of the less-studied species in terms of research and only a few experiments have been done.

Since citrus is prominent cash generating crop in more than 55 districts of Nepal (MOAD and FAO, 2011), it is the backbone of agricultural GDP (Acharya *et al.*, 2011; Adhikari and Rayamajhi, 2012). In 2012, the government of Nepal has signed a trade agreement with the Chinese government especially for the export of mandarin and sweet orange from Syangja and Sindhuli districts respectively (Sharma *et al.*, 2015). The latest status reveals that Nepali citrus production is increased by 4.81% per annum (World Data Atlas, 2017), nevertheless an unprecedented invasion of the CCF has hit farmers hard (Bhandari and Upreti, 2018). Due to this destructive pest, both the loose skinned and tight skinned citrus posed threats. Despite the potential of export (NHPC, 2017), the current practices of management seem weak and liable to the inability to meet the quality demands from inside and outside the borders. Therefore, this study aimed to make a comprehensive review of ongoing novel management techniques of the CCF, both in Nepal and across the globe so that following the control measures, our citrus industry be safeguarded against the invasion of the CCF in the future.

## MATERIALS AND METHODS

To prepare this article, we reviewed a series of papers published in various journals, visited several websites and included varieties of documentations from any reliable sources while some information was from our observations and experiences.

## RESULTS AND DISCUSSION

### Life cycle

The *Bactrocera minax* is a peculiar of the genus *Bactrocera* in several aspects. Firstly, it is an oligophagous restricting to citrus host only (Allwood *et al.*, 1999) and a univoltine species with a comparatively longer period of overwintering (about 5-6 months). Secondly, it is a cold-tolerant species prevalent in cold regions. It survives with a strategy of reduced respiration rate even in soil with higher water content (Wang *et al.*, 2019). Thirdly, its larvae are greater than that of other Tephrid flies, which ranges between 16-24 mm (Xia *et al.*, 2018). The life cycle and phenology of the CCF are crucial to devising a control-measure tactic against *B. minax* (Dorji *et al.*, 2006).

In Nepal, the CCF emerges by mid-March to late April, however it depends upon the local temperature, elevation and other climatic factors (Chauhan *et al.*, 2019). After emergence, adults live by honeydew secreted by aphids on the nearby woods and in the month of June-July, female CCF attacks young citrus fruits of diameter 2-4 mm and deposits 50-750 eggs. However, 11 mm is reported to be the most susceptible diameter in mandarin (*Citrus reticulata* Blanco) (Schoubroeck, 1999). Eggs last roughly a month and develop into larvae. For two months, the larvae feed the pulp until matured 3rd instar stage and with attacked fruits dropping off; it goes to the soil for overwintering as a resting pupa even the deepest to 45 cm. The pupal stage is the longest stage that lasts for 150-200 days. Some literature claim that the *B. minax* diapause is the weakest one that can be broken with a long-duration chilling temperature (Dong *et al.*, 2013).

### Distribution of CCF in Nepal

Though Nepal has developed a survey protocol for Citrus flies, the CCF distribution is reported from very few places precisely (NPPO, 2019). The CCF is found to be spread from eastern Nepal (NCRP, 2014) to Lamjung district in the west (Adhikari *et al.*, 2019). Based on the few previous pieces of literature and own observations, we speculate that the CCF is spread to even far than Syangja district on the western side.

### Host range

The CCF is oligophagous species restricted in citrus hosts (Allwood *et al.*, 1999; Dong *et al.*, 2014). As there's no effective technique of trapping for the CCF, only the infested fruit seems reliable means of measurement of severity (Xia *et al.*, 2018). Literature from China suggests the higher rate of the severity of infestation in tight skinned citrus species like Navel orange (*Citrus sinensis* Osbeck) near to 100%, however the loose skinned: mandarin (*Citrus reticulata* Blanco) is also posed with highest of 74.7% infestation. While in Bhutan more than 50% infestation is common in mandarin (*Citrus reticulata* Blanco) orchards (Dorji *et al.*, 2006).

This is supported by the behavioral fact that the female fly pierces up to pulp (eucarp) in tight skinned fruits unlike only outer peel in loose skinned citrus, the invasion is comparatively less severe in mandarin (Schoubroeck, 1999). A similar case is prevalent in Nepal. As Sweet Orange (*Citrus sinensis* L. Osbeck) is mostly grown tight skinned citrus fruit, it has been affected by the CCF in greater infestation proportion than Mandarin (*Citrus reticulata* Blanco). While field survey revealed that acid lime and lemon cultivation has vanished in several pockets due to the greater infestation rate than sweet orange (Table 1, 2).

### Management approaches

In the management of fruitflies, prevention is one of the most effective strategies to look for (Dias *et al.*, 2018) In achieving so, monitoring is crucial (Enkerlin *et al.*, 2017). Identification is the key step for monitoring. In Nepal, fruit fly identification is performed manually by few specialists through morphological analysis of species.

### Monitoring experiments

This univoltine fruit fly can be monitored by the use of different techniques such as emergence-bottle monitoring, bait-monitoring trap, dry-lure trap and fruit mimic balls (Schoubroeck, 1999).

### Emergence-bottle monitoring

In Nepal, Chauhan *et al.* (2019) used a plastic bottle of size 30 cm×30 cm filled with sandy loam soil to rear maggots from attacked sweet orange fruits. Some pioneers have used nets to cover the rearing bottles filled with sterilized sand beneath sweet orange plants also (Adhikari and Joshi, 2018; Adhikari *et al.*, 2020).

**Table 1.** Host Range of CCF.

| Common name | Scientific name | Highest infestation rates (%) reported |
|---|---|---|
| Navel Orange | *Citrus sinensis* Osbeck | 100 |
| Ponkan | *Citrus poonensis* Hort. ex Tanaka | 50 |
| Mandarin | *Citrus reticulata* Blanco cv. Tankan | 74.7 |
| Bingtang Orange | *Citrus.sinensis* (Linn.) Osbeck | 70-80 |
| Dahong Orange | *Citrus sinensis* (Linn.) Osbeck cv. Da Hong | 72-75 |
| Jinch Orange | *Citrus sinensis* (Linn.) Osbeck cv. Jincheng | 1.7 |
| Early Ripening Satsuma mandarin | *Citrus unshiu* Marcovitch | 73.2 |
| Pomelo | *Citrus maxima* | 71.4 |
| Sour Orange | *Citrus aurantium* L. | 99.3 |

**Table 2.** Reported Host of CCF in Nepal.

| Common Name | Scientific Name | References |
|---|---|---|
| Sweet orange | *Citrus sinensis* L. Osbeck | (Chauhan *et al*., 2019; Adhikari *et al*., 2019) |
| Lemon | *Citrus limon* L. Osbeck | (Adhikari *et al*., 2019) |
| Lime | *Citrus aurantiifolia* | (Adhikari *et al*., 2019) |
| Mandarin | *Citrus reticulata* Blanco | (Adhikari *et al*., 2019) |

### Dry-lure trap

The dry-lure trap is usually adopted a week before the probable emergence. In April-July, the higher, 53% of total emerged female flies were caught in a dry lure trap made of cotton wicks, fixed inside the plastic can with 4 holes of 2 cm diameter, soaked in a watery solution of 10% Protein Hydrolase (PH), 0.4% malathion 50EQ and 0.1 % Sandovit™ detergent (Schoubroeck, 1999).

### Fruit mimic balls

The fruit mimic balls experiment is known in Nepal. On testing of 15, 22, 35 mm size of fruit mimic balls with non-drying glue during oviposition in Bhutan, most flies were attracted to a green ball of size 22 and 35 mm than 15mm (Schoubroeck, 1999). Another experiment showed that the orange or green-yellow mixtures colored spheres were more efficient than similar-sized red, yellow, green, blue, black, or white spheres of 50mm (Drew *et al*., 2006). However, this technique seems impractical and less standard for villages (Schoubroeck, 1999).

## Prevention of CCF

### Orchard sanitation and shallow tillage

Clearing off the dropped and hanging attacked sweet orange fruits and packing off in air-tight plastic bags to kill maggots is a common practice in orange groves in Nepal and often suggested to farmers (Adhikari and Joshi, 2014). Some Chinese literature suggests that good sanitation practices can significantly reduce the infestation from 50-100% to below 1%. As the majority of overwintering pupae rests at depts 4-6cm, raking or shallow plowing will expose pupae to natural enemies like birds (Xia *et al*., 2018). However, experiments from Bhutan reported quite different a result. Schoubroeck, 1999 argued that soil tilling hardly adds up to the natural control mechanism, puparium might sometimes bury deep down to 45 cm. Somehow tilled orchards become good shelter opportunity for pupae to remain as the natural control system is destroyed, probably ants, spiders or braconid wasps are killed in fall/winter tillage. Several reports have found that matured 3rd instar larvae move to the soil within 7 days of fruit drop and goes to overwintering (NCRP, 2014). In Nepal, most of the orchards are at sloppy lands, which increase the chance to roll off dropped fruits to bushes. So, sanitation before 7 days of the fruit fall is quite tedious and non-economical. Besides, the matured 3rd instar maggots inside the fruit make a hole in the rind of the fruit and jump in the ground below the tree for pupation in the soil.

### Use of parasitoid

The use of natural enemies is still unknown in *B. minax*. The braconid wasp, *Fopius arisanus* (Sonan) is potentially used in area -wide control against *Ceratis capitate* (Vargas *et al*., 2009). In *B. minax* some ants, spiders were reported from China (Xia *et al*., 2018). Van Achterberg, 1999 reported some parasitoids like *Diachasmimorpha feijeni* (Hymenoptera: Braconidae) on the CCF pupae in the soil. Since eggs grow up in fruits, in *B. minax* there's no parasitoid to limit the number of eggs or larvae (Huasong *et al*., 1998).

### Use of entomopathogenic fungus

We didn't find enough literature about the use of entomopathogenic fungus against *B. minax* but some larvae of other multivoltine species like, *C. capitate*, *B. dorsalis*, *B. zonata*, *B. cucurbitae*, *B. carambolae* were tested against pathogenicity of different strains of entomopathogens like *Micorhizium anisopliae* and *Beauveria bassiana* in sand (Ekesi *et al*., 2002; Toledo *et al*., 2006; Sookar and Bhagwant, 2010; Sachin, 2012; Brito *et al*., 2019). Huasong *et al*., 1998 reported that *Beauveria tenella* is one of the entomopathogenic fungi to be used against *B. minax*. In 2002, Ekesi *et al*., 2002 suggested that the combined use of soil application of *M. anisopliae* and GF-120+spinosad bait spray is an effective IPM strategy for field suppression of *B. invadens* on mango (Figure 1).

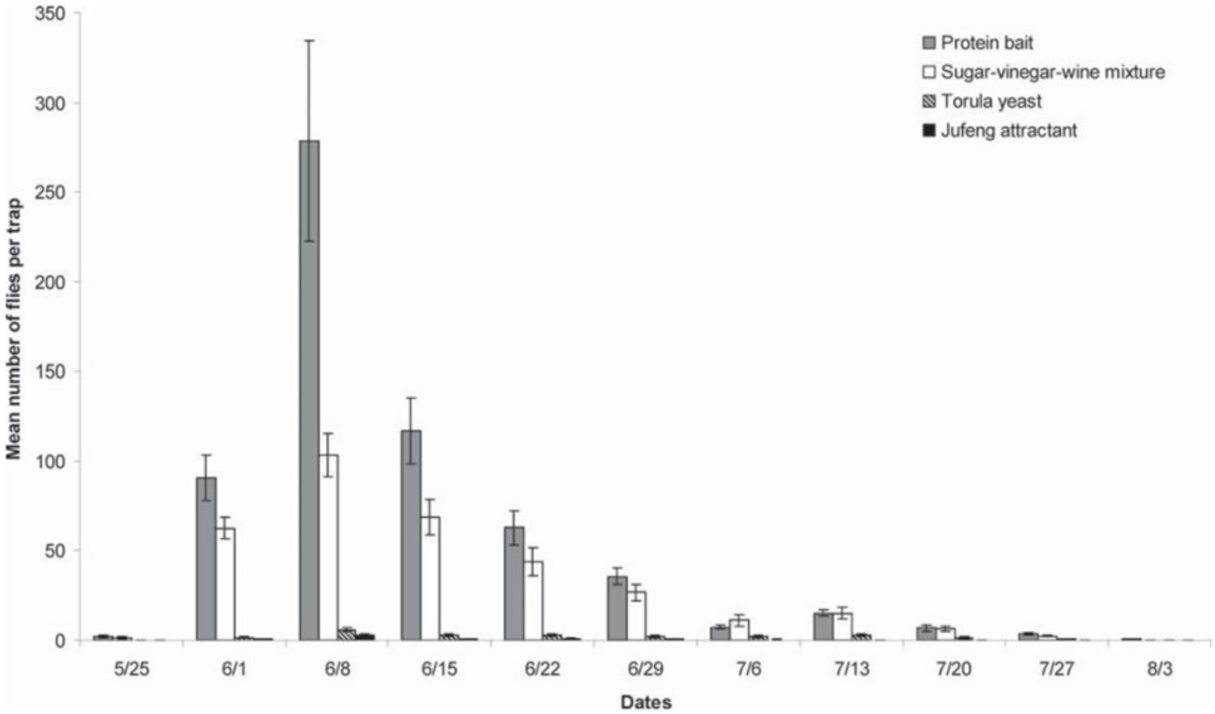


**Figure 1.** *Mean number of B. minax flies (mean ± SEM) captured per trap weekly using various lures (H-protein bait, SVW, torula yeast, and Jufeng attractant) in experimental orchards in Hubei province, China (Zhou et al., 2012).*

### Control with natural product insecticides

Some evidences hint the possible use of natural product insecticides like Neem Seed Cake (NSC) and parasitoid against larvae of some fruitflies (Singh, 2003; Silva *et al.*, 2013). In *Ceratis capitata* both NSC and parasitoid *Diachasmimorpha longicaudata* (Ashmead) increased the larval mortality and decreased the adult emergence (Alvarenga *et al.*, 2012). NSK only, however is not so effective to be used as toxic bait against *C. capitate* (Silva *et al.*, 2013).

### Mass trapping

### Use of food-based lures

Since two common tephrid parapheromone lures: cuelure and methyl eugenol did not affect *B.* minax, food-based lures are the choice. These lures are used for trapping only while insecticides are mixed to kill the fly. In China, the common food-based lures include hydrolyzed protein (H-protein bait), homemade bait made from beer yeast, torula yeast, sugar-vinegar-wine (SVW), commercial protein baits: Jufeng (Zhou *et al.*, 2012). The H-protein, proved to be efficient in attracting more no. of flies among these four treatments.

The efficacy of these lures is quite inconsistent. However, most studies had evidence of Great fruit fly bait (a commercial protein bait manufactured by Ecoman Biotech Co. Ltd.), Jufeng and 20% hydrolyzed protein are superior and are statistically at par. Though 20% hydrolyzed protein caught more no. of flies. It is also reported that 1:2 (Jufeng: water ration) solution had the most attractive effect on *B. minax* than 2:1 solution, 100% Jufeng solution or Sugar-Vinegar-Chinese liquor mixture.

In Bhutan Mahat *et al.* (2016) reported a similar effect of hydrolyzed protein. Pinnacle protein was found to be the most effective among tested four treatments: Pinnacle protein Probiofer L, Probiofer A and Jaggery for both flies capturing (Table 3) and killing flies while using with Spinosad (Table 5). The field reports by Xia *et al.* (2018), in China, suggests that 5% Orange + 5% H-protein + 5% Chinese liquor had a superior effect overall nine treatments (Table 4).

### Sterile Insect Technology (SIT)

SIT, as a part of Area-Wide Control of Chinese citrus fly, in China showed reduced the CCF infestation from 7.5% to 0.005% when 5600 and 95000 male flies irradiated with Gy of 60Co rays were released in the ratio of 12.5:1 and 45:1 in 1987 and 1989 respectively (Huasong *et al.*, 1998). Though SIT, once was given the national priority in China, it seemed very costly and not recommended as it has more technical obstacles to rearing larvae of *B. minax* due to univoltine nature (Xia *et al.*, 2018). In the context of Nepal, SIT is not so economical and applicable.

### Area Wide Control Program (AWCP) of Pest

Area Wide Control Program (AWCP) of Pest is a large domain Integrated Pest Management (IPM) approach to reduce the fly population. It integrates biologically based pest technology into an IPM package that is economically viable, environmentally friendly and sustainable (Mau *et al.*, 2007; Vargas *et al.*, 2008). AWCP in Hawaii, US, in 2007 had the operational, research, education and assessment components. While education and assessment components included: population monitoring, field sanitation, application of protein bait sprays, male and female annihilation with male lures and other attractants, sterile insect releases, conservation or release of beneficial parasitoids (Mau *et al.*, 2007).

**Table 3.** Mean number of *B. minax* captured in PET bottle traps containing different lures in Tsirang, Bhutan (Mahat *et al.*, 2016).

| Lures | Mean flies captured* |
|---|---|
| Pinnacle protein | 45.12a |
| Probiofer L | 42.64a |
| Probiofer A | 11.52b |
| Jaggery | 5.44b |

*Statistical significance at P < 0.05 [Means followed by the same letter do not differ significantly] [Fisher's LSD test on log (x+1) transformed data; P < 0.05].

**Table 4.** Result of field lures used in China (Xia *et al.*, 2018).

| Lures | No. of *B. minax* (entire season)* |
|---|---|
| 5% H-protein | 82.7 cd |
| 5% Sugar | 76.0 d |
| 5% Sugar + 5% Chinese liquor | 63.6 e |
| 5% Sugar + 5% Vinegar | 77.0 d |
| 5% Sugar + 5% H-protein | 103.0 c |
| 5% Sugar + 5% Orange Juice | 82.3 cd |
| 5% Sugar + 5% Vinegar + 5% Chinese liquor | 90.0 c |
| 5% Vinegar + 5% Chinese liquor + H-protein | 179.7 b |
| 5% Orange + 5% H-protein + 5% Chinese liquor | 273.7 a |
| 5% Sugar + 5% Orange Juice + 5% H-protein | 256.3 a |
| 5% Sugar + 5% Vinegar + 5% Chinese liquor + 5% H-protein | 141.3 bc |

* Statistical significance at P < 0.05.

**Table 5.** Mean number of *B. minax* killed with protein baits applied as spot sprays on mandarin trees in Tsirang, Bhutan (Mahat *et al.*, 2016).

| Lures | Mean flies captured* |
|---|---|
| Pinnacle protein | 23.26a |
| Probiofer L | 4.00b |
| Probiofer A | 6.63b |
| Control | 0.00b |

* Statistical significance at P < 0.05; Source: (Mahat *et al.*, 2016) [Means followed by the same letter do not differ significantly] [Fisher's LSD test on log (x+1) transformed data; P < 0.05].

In Nepal, Junar Superzone, Sindhuli under Prime Minister Agriculture Modernization Project (PMAMP), in May to July 2018, implemented the AWCP with the major components: the use of protein baits named Great fruit fly bait (Protein hydrolysate 25+0.1% Abamectin) as spot application underside of the 0.5 to 1 m2 leaf for 10 times at a weekly interval as per the protocol developed by Ecoman Biotech, China and the field sanitation. Interestingly, the average fruit loss due to the fly decreased to 10.90% in 2018 from 56.7% in 2017. While 6% in 10.90% infestation was due to factors other than the CCF, water stress, nutritional disorders and bug damages (Acharya and Adhikari, 2019; Adhikari *et al.*, 2020).

## Precautions and community awareness

A simple, yet a crucial step is precaution and community awareness to control spread of the CCF. Though an adult fly has got a potential to take flight upto 1 km, it needs a strict precaution measures to limit the fly spreads. Several hording boards, caution signs and even fines for transfer of infested fruits had been taken into consideration, in AUS for the purpose of safeguarding an uninfested area famously known as the Fruit Fly Exclusion Zone (FFEZ) (Jessup *et al.*, 2007) (Figure 2).


**Figure 2.** *Precautionary road signs on highway into the Fruit Fly Exclusion Zone (FFEZ) in New South Wales, Australia, a strategy to reduce the amount of fruit fly-infested fruit entering the FFEZ (Source: Jessup et al., 2007).*

## Conclusion

The Chinese citrus fly (*B. minax*) is havoc in the Nepali citrus industry, especially in tight skinned citrus fruits. Nepal, apart from a few domestic prevention measures, is yet to receive AW-IPM or AWCP. While devising the AWCP, individual components have a significant role. Clearing off the dropped citrus fruit soon after dropping and tillage practice before the spring contribute less to the total no. of fly that emerges in the spring. Monitoring the emergence of fly in a contained local plastic bottle was found to be popular. Therefore, attracting female fly during the oviposition period (May-August) with 25% protein hydrolase or commercial Great Fruit fly bait and subsequent killing would be an effective component in AWCP. Similarly, key awareness about infestation and checking the transport of infested fruits across borders would be effective to restrict the fly. While the use of natural enemies, parasitoid, sterile insect technology (SIT) seemed less frequent and the latter is not so applicable in Nepal.

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