

Fruit Flies in Citrus Fruits with Special Reference to Chinese Citrus Fly, *Bactrocera minax* (Enderlin) (Diptera: Tephritidae): Status and Management Options in Nepal

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Abstract

Fruit fly is one of the serious pest of *Citrus*. Among 16 species of fruit fly reported in Nepal, 6 species of fruit flies were found in citrus fruits. According to pest surveys done in Dhankuta and Sindhuli, the species found are: Melon fly (*Bactrocera cucurbitae*), Oriental fruit fly (*B. dorsalis*), Peach fruit fly (*B. zonata*), *B. tau*, *B. scutellaris* and *Dacus longicornis*. Chinese fruit fly, *B. minax* (Enderlein) (Diptera: Tephritidae) has created devastation in the sweet orange orchards in eastern hills of Nepal and the infestation rate have been found up to 100%. Unlike other *Bactrocera* species, *B. minax* is univoltine and oligophagous and adult flies becomes active from April to July. The two highly attractive para-pheromones, Cue lure and Methyl Eugenol used for many *Bactrocera* species are not attractive for this species. For *B. minax*, food based lure i.e. protein baits are used. Maintaining field sanitation by proper disposal of infested fruits, winter/summer ploughing, visual cue lures such as colored sticky spheres, mass trapping by using para-pheromones and food based lures, foliar insecticidal spray are the pest management strategies adopted in China, Bhutan, Sikkim and Nepal. Area Wide control program has been launched using technologies: monitoring, Great Fruit Fly Bait (Protein Hydrolysate 25%+0.1% Abamectin) developed by Ecoman Biotech, China, trapping and field sanitation in the year 2018 which has proved successful in control of *B. minax* in the sweet orange orchards of Sindhuli.

Keywords: Fruit Fly; *Bactrocera minax*; Protein Baits, Quarantine Pests

Introduction

Citrus is indigenous and currently most widely cultivated fruit crop of Nepal. Citrus species occupy more than 32% of the total fruit area of Nepal. Citrus is cultivated in the area 46, 328 hectare (ha) land with production and productivity being 239,773 Metric tonnes (Mt) and 8.96 Mt/ha respectively [1]. Mandarin and Sweet Orange is mostly cultivated in the mid-hills while acid lime/lemon is mostly cultivated in the lower altitude areas in the country [2].

In terms of export, citrus fruits share 3% by volume and share is 16% by volume and 14% by values in terms of imports [3]. Fruit fly species, which can fly several kilometers within an hour, have been enlisted as quarantine pests for China according to bilateral trade protocol signed between Nepal and China in 2012 [4]. Among the species, Chinese Citrus Fly (CCF), *Bactrocera minax* have created a havoc to the citrus fruit industry of Nepal [5]. Several studies have been carried out in China [6], Bhutan [7,8] and Nepal [5,9,10,11] on

CCF. Less reviews have been carried out on the pest. Thus, this paper aims to perform the comprehensive review on the phenology, pest biology and management techniques adopted worldwide for the eradication of the pest and safeguard of Citrus fruit industry in Nepal.

The fruit flies

Fruitflies belonging to Dipteran family Tephritidae (= Trypetidae), are of great economic importance occurring in fruits and fruit vegetables. There are about 4000 species (including subspecies) in 483 genera known around the world [12]. There are various types of fruit flies found in the world. Phytalmine fruit flies, belonging to subfamily Phytalminae; Dacine fruit flies (Subfamily Dacinae) including *Bactrocera* and *Dacus*; Ceratidine Fruit Flies (Subfamily Ceratitidinae); Trypetine fruit flies (Subfamily Typetinae) and Tephritine Fruit flies (Subfamily Tephritinae), also known by the highest number of species [13]. A number of species infest a wide variety of fruits, vegetables, flowers, heads, seeds, leaves and other plant parts [14]. They are generally polyphagous, having higher rate of fecundity and ability of quick spread [15]. Their distribution is cosmopolitan i.e. they are found in tropical, subtropical and temperate regions [16].

Fruit flies in Nepal

Fruit Flies are one of the world's most destructive horticultural pests that deplete the quality and quantity of vegetable and fruit trees [17,18]. In Nepal, fruit flies are the major pests for cucurbits and citrus crops. A study conducted to estimate the fruit fly population and identify the fruit flies in Kathmandu and Kavre (1994 to 1996) reported six different species of fruit flies namely *Bactrocera cucurbitae*, *B. dorsalis*, *B. zonata*, *B. tau*, *B. scutellaris* and *B. yashimotoi* [18]. Later, six reference fruit fly species were collected and stored at Entomology laboratory of Fruit Development Directorate, Kirtipur [4]. At present, there are 17 species of fruit flies' species in Nepal according to various surveys done at various places. Previously, there were 12 species but five other species were identified during training on fruit fly identification conducted by Plant Protection Directorate, Hariharbhawan, Kathmandu in 2015 at Entomology Division of NARC, Khumaltar [4,19]. These are: *B. dorsalis*, *B. zonata*, *Bactrocera correcta*, *B. cucurbitae*, *B. tau*, *B. scutellaris*, *Bactrocera diversus*, *Bactrocera caudatus*, *Bactrocera minax*, *B. yashimotoi*, *Dacus longicornis*, *Bactrocera nigrofemoralis*, *Bactrocera latifrons*, *Bactrocera aritfacies*, *Bactrocera tuberculata*,

Dacus ciliates and *Bactrocera tsuneosis*. Among these species, *B. tsuneosis*, collected from Sweet Oranges of Helambu, Sindhupalchowk (1984) doesn't exist in Nepal due to mistake in identification, which was taxonomically corrected as *B. minax* later [19].

Fruit fly in citrus fruits of Nepal

Citrus fruit flies are the most serious insect pest in sweet orange and lemon in the eastern hills of Nepal. Pest surveys were conducted in citrus orchards of Sindhuli and Syangja in the year 2012-13 involving experts from different sectors revealed two species of fruit flies i.e. *B. cucurbitae* and *B. dorsalis*. The surveillance in selected Sweet Orange Orchards of Sindhuli (May-Dec., 2014) revealed 5 species of fruit fly in Methyl Eugenol trap namely *B. cucurbitae*, *B. dorsalis*, *B. zonata*, *B. tau* and *B. scutellaris* and six species in Cue lure (including *D. longicornis*) [17]. During the study by [17], maximum number of *B. dorsalis* was trapped in Methyl Eugenol followed by *B. zonata*, *B. tau*, *B. scutellaris* and *B. cucurbitae*. Similarly, Cue Lure trap followed by *B. dorsalis*, *B. cucurbitae*, *B. scutellaris*, *B. zonata* and *D. longicornis*. Similar study conducted by NCRP, Dhankuta from September 21, 2014 to September 16, 2015 also reported the highest number of *B. dorsalis* trapped in Methyl Eugenol Trap and *B. tau* in Cue lure [19]. McPhail trap, set up using autolyzed protein in experimental orchard of NCRP, Dhankuta revealed the trapping of maximum number of *B. minax* along with *B. dorsalis* and *B. tau* [19].

Figure 1: Life cycle of *B. minax* with damage symptom (Source: en.ecomanbiotech.com).

Chinese citrus fly (CCF) (*Bactrocera minax*, Enderlein)

Chinese citrus fly (CCF) is a dangerous species causing great losses to citrus fruits like sweet orange, hill lemon and mandarin too in eastern hills of Nepal [20,21]. *B. minax* is different from other species in the genera *Bactrocera*. Generally, fruit fly species are polyphagous and multivoltine but *B. minax* is oligophagous (feeds only on citrus plants) and univoltine (produces one generation per year). It is also larger in size, larva can be as long as 16 mm and adults can reach as long as 24 mm. In adults, dorsal part of thorax consists of three yellow patches and abdomen is long as compared to other fruit fly species [22]. *B. minax* is among the cold tolerant species in the genus *Bactrocera* [23].

Life cycle

In Nepal, Adults start to appear from April. Months of May to July is very much favorable for oviposition in smaller fruits by *B. minax* [19,24]. Female lays eggs 15 days or so after mating. Eggs inside the fruit hatch into larvae (called Orange Maggots) in about 90 days and develop as fruit ripen. Fruits with maggots inside turn yellow earlier than normal fruits and fall down. High infestation has been observed between first to third week of July [25]. Larvae then crawl out and burrow into the soil 4-6 cm deep to pupate. Larval stage lasts roughly for two months. The pupal stage is the longest stage lasting from 150-200 days [26].

Month	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Adult				■	■	■	■	■				
Egg				■	■	■	■	■				
Larva						■	■	■	■			
Pupa	■	■	■	■	■					■	■	■

Table 1: Life cycle of *B. minax* in Nepal [11].

Origin and distribution

B. minax is likely to be originated from Yunnan-Guizhou Plateau of China [31]. It was then later found in China (Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Shaanxi, Sichuan, Yunnan) [32], India (West Bengal, Sikkim), Bhutan [29]. Adult flight and transport of infested fruits are the means of movement and dispersal in uninfested fruits. Many *Bactrocera* spp. including *B. minax* can fly 50-100 km [34]. *B. minax* might have dispersed in eastern hills of Nepal from Bhutan through Sikkim [8,9].

Host preference

All cultivated citrus species are hosts of *B. minax*. Higher fruit infestation rates (up to 100%) are reported in Navel Oranges (Sweet Oranges) than other species followed by Mandarin, Early Ripening Satsuma, Pumelo [28]. *B. minax* appears to prefer laying eggs on fruits of 2-4 cm diameter. Fruit sizes of sweet oranges usually fall within this range during oviposition period. *B. minax* may choose to oviposit in thin skinned fruits. This mechanism of host preference has been proved in other tephritid flies [28]. The losses by *B. minax* has been reported up to 97% in sweet oranges and up to 15% in mandarins in eastern hills of Nepal [21].

Damage

Fruits infested with fly larvae falls from the tree. Damage by larvae tunneling in fruits and feeding the pulp varies with the type and maturity of fruit, the number of larvae and environmental conditions. By the time maggot reaches the 3rd instar, a large portion of fruit gets destroyed. Heavily infested fruits often drop before harvest causing a great loss to citrus grower [8,11].

Fruit fly management strategies

Field sanitation and fall/winter soil treatment

This practice is the must for any citrus orchards. The fallen and hanging infested fruits are collected in large bags. These bags with infested fruits are subjected to various treatments like heat treatment (exposing the bags under the sun), boiling, deep bury (at least 30 cm below the soil surface), burning, putting the infested fruits in shallow pond [31,32]. Studies have indicated that good sanitation practices can significantly decrease *B. minax* infestation [33]. As *B. minax* pupae overwinter in the soil at the depth of 4-6 cm, Raking and/or shallow plowing in fall/winter can expose the pupae to outer temperature, soil insecticides and natural enemies like birds [33,34].

Bagging

Fruit bagging is one of the most effective methods to control pests and protect fruits which not only has the control effect reaching (or near) 100% but also has small effect on fruit quality. Bagging should be conducted during young fruit period or before color changing and application of pesticides should be done 3-5 days before bagging. Fruit Bagging has small impact on sugar degree of citrus and it is better to select white paper bags as bagging materials [6].

Use of colored sticky sphere balls

Sticky spheres and sticky cards can also be used. It was found that *B. minax* was more attracted to sticky sphere balls colored with orange or green-yellow mixture than to similar spheres with different color and to discs with same color/combination [8].

Para-pheromone traps

Fruit flies are monitored by using Para-pheromone Traps, which works as "attract and kill" [35,36]. Methyl eugenol and cue lure, 2 potent male attractants for many *Bactrocera* species, were reported to not be attractive to *B. minax* [37].

Food based lures (Bait Spray)

The most common food-based lures in China are hydrolyzed proteins, waste brewer's yeasts, sugar + vinegar mix [38-40] reported no statistical difference in attractive efficacy between 20% hydrolyzed protein (20% H-protein lure), Great Fruit fly lure (a common commercial protein based product in China), Jufeng Fruit fly lure, Conventional Sugar-Vinegar-Chinese liquor (Conventional SVL) and improved Sugar-Vinegar-Chinese liquor (Improved SVL, sugar+acetic acid+ethanol) although 20% H-protein had the highest trapped flies. A study conducted in NCRP Paripatle, Dhankuta to find the efficacy of various protein bait spray revealed Beer Supernatant and debris+honey had highest number of flies caught in McPhail trap and was significantly different from Beer Supernatant and Debris, Beer Supernatant only and Sofri Protein but wasn't significantly different from Hydrolyzed Protein Treatment [24]. Based on various experiments, Combination of methyl eugenol and hydrolyzed protein bait spray is recommended in Dhankuta while Hydrolyzed protein bait spray is recommended in May to July months in Sindhuli district [5,24]. Malathion is the usual choice insecticide for fruit fly control and this is usually combined with protein hydrolysate to form a bait spray. Bait sprays work on

the principle that both male and female tephritids are strongly attracted to a protein source, as protein is essential for the reproduction and vitellogenesis. Bait sprays have the advantage over cover sprays in that they can be applied as a spot treatment so that the flies are attracted to the insecticide and there is minimal impact on natural enemies [26,41].

Foliar pesticide spray

Foliar Spray is considered is one of the most common *B. minax* management options in China. Commonly used insecticides are organophosphates such as trichlorfon and/or pyrethroids such as deltamethrin [41,42]. Organophosphate pesticides: Phoxim and Chlorpyrifos was found to have strongest influence on pupation and emergence of the pest respectively but their use should be minimized due to their high toxicity and long residual period [43].

Sex pheromone and citrus plant chemicals

Female rectum extracts had the highest attraction to both males and females as compared to three other lures (Male Rectum Extracts, Hydrolyzed protein and sugar solution with wine vinegar mixture) [44]. Similarly, Citrus plant volatiles play an important role in host finding and oviposition behavior of *B. minax* and female adults were attracted to nonanal, citral, limonene and linalool from citrus fruits [45].

Sterile insect technique (SIT)

SIT technique was successful in reducing *B. minax* attack on oranges from over 5% to around 0.1% in Guizhou, China during the year 1993-94. Sterile males were produced by collecting infested fruits in late larval stage, placed in containers with sand to pupate and treated with irradiation [46].

Area wide control program (AWCP) in Nepal

AWCP has been implemented for the first time in Nepal to manage Chinese Citrus fly in sweet Orange Orchards of Sindhuli. This program was conducted by the joint effort of PMAMP, Program Implementation Unit (Junar Super Zone, Sindhuli), Karma Chemical Company Pvt. Ltd., Kathmandu, Ecoman Bio-tech China and sweet Orange growers of Sindhuli from April to July, 2018 in 100 Acres orchards. It included technologies: Monitoring, Protein Bait Spray (Great Fruit Fly Bait), trapping and field sanitation. Bait spray of Great Fruit Fly Bait (Protein hydrolysate 25% and 0.1% Abamectin): 1 part in 2-part water at the rate of 50ml/0.5-1m² underside of

the leaves in one among three trees at weakly interval for 10 times. Fruit Loss due to CCF decreased from 56.71% in 2017 to 10.89% in 2018. AWCP has proven successful in suppressing CCF havoc in Sindhuli [10,47].

Recommendation of management strategies

Among various Fruit Fly management strategies recommended by plant doctors in Plant Clinics session from September 2013 to July 2016, maximum (93.33%) recommended para-pheromones followed by sanitation (70.55%), Chemicals (26.11%), Botanicals (24.44%), Cultural Methods (22.22%), Pest Exclusion Measures (6.11%) and Food lures (4.44%) [48].

Criteria	Measures
Economic	Sanitation, Cultural, Botanicals
Effective	Para-pheromones, Sanitation, Exclusion
Safe	Para-pheromones, Botanicals, Exclusion
Practical	Para-pheromones, sanitation, Botanicals
Locally Available	Sanitation, cultural, botanicals

Table 2: Criteria for Recommendation of FF management strategies recommended in Plant Clinic Sessions from September 2013 to July 2016, Nepal [48].

Conclusion

Fruit fly is one of the major pests devastating the horticultural crops including citrus with 6 fruit fly species reported in Nepal. *B. minax* have been found in Sweet Orange Orchards in eastern hills of Nepal with infestation rate found up to 100%. Various studies indicated no single and independent management strategy have been successful for fruit fly species. Food based lure with protein hydrolysate (Protein Bait containing insecticides) has been found efficient for monitoring and controlling *B. minax*, rather than Pheromone traps. Area Wide Control Program, proven to be successful in Sindhuli, Nepal, should be implemented for efficient control of *B. minax*.

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