

Trapping of Chinese Citrus Fruit Flies (*Bactrocera minax*) using Protein Bait in McPhail Traps and Pet bottle

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Abstract

Mandarin orange (Citrus reticulata) is one of the most important fruit crops in Bhutan, both as a major export commodity and a critical source of rural income. However, its production is severely threatened by the citrus fruit fly, Bactrocera minax (Diptera: Tephritidae), which causes substantial fruit drop and economic losses. This study monitored and analysed the population dynamics of B. minax females using protein hydrolysate bait in McPhail traps and Pet bottle traps across three orchards located in Dagana and Tsirang Dzongkhags from April to mid-August 2025. The results showed clear variation in fruit fly catches between locations, trap types, and sampling rounds. McPhail traps consistently recorded higher mean catches compared to PET bottle traps, indicating their greater efficiency in monitoring female populations. Location B exhibited the highest fruit fly densities, with peak captures reaching six flies per trap during the mid-season round, while Locations A and C recorded relatively lower levels. Interestingly, despite good orchard sanitation practices in Location C, fruit fly counts remained high, suggesting that microclimatic conditions such as temperature and humidity strongly influence population dynamics. Overall, fruit fly activity was lowest during the early season, increased significantly in the mid-season rounds, and declined toward August, reflecting seasonal fluctuations in fly emergence and activity. These findings highlight the role of both orchard sanitation and environmental factors in shaping B. minax populations. Importantly, the study demonstrates the potential of protein bait trapping as a practical management tool and provides baseline data for developing integrated pest management (IPM) strategies tailored to local conditions.

Key words: Citrus; *B. minax*; Traps; Protein bait; Monitoring

1. Introduction

Mandarin orange (*Citrus reticulata* Blanco) is one of the most important citrus fruits globally, valued for its nutritional content, commercial significance, and consumer demand. Citrus fruits, including mandarins, contribute significantly to global fruit production and trade, with total world citrus production estimated at ≈ 158 million tonnes in 2022, of which mandarins and tangerines accounted for nearly 40 million tonnes (FAO, 2023). These fruits are not only a vital source of vitamins, minerals, and antioxidants but also play a major role in the livelihoods of millions of farmers worldwide. Their economic importance is reflected in their widespread cultivation across Asia, South America, and the Mediterranean, where mandarins are cultivated both for domestic consumption and international markets (Liu et al., 2020; FAO, 2023).

In Bhutan, mandarin is regarded as one of the most commercially significant fruit crops and a key source of rural income. Citrus, especially mandarin, is widely grown across the mid-hills between 800–1,600 meters above sea level, where agro-climatic conditions are favorable (Dorji, 2007). According to the National Statistics Bureau (NSB, 2023), mandarin accounts for the largest share of Bhutan's fruit exports, contributing substantially to the country's agricultural GDP and foreign exchange earnings. The crop also plays a vital role in household livelihoods, as many smallholder farmers depend on mandarin cultivation as a primary source of cash income. However, productivity is often constrained by factors such as fruit fly infestations, poor orchard management, citrus decline disease, and limited adoption of improved post-harvest handling practices (Wangchuk et al., 2017). Despite these challenges, mandarin remains central to Bhutan's horticulture sector and rural food security, making it a priority crop for sustainable orchard management and pest control interventions.

Insect pests are one of the most significant constraints to mandarin orange production, with the citrus fruit fly, *Bactrocera minax* (Enderlein), considered the most destructive species in high-altitude citrus-growing regions of Asia (Dorji et al., 2006). Unlike most tephritid fruit flies, *B. minax* is univoltine, producing only one generation per year, but its synchronized lifecycle allows mass infestation of citrus fruits during their critical developmental stages (Wang et al., 2019). Yield losses attributed to *B. minax* are substantial, with unmanaged orchards reporting fruit damage levels ranging from 50% to 80% (Xia et al., 2021). Infestation occurs when adult females

oviposit into immature fruits, and the developing larvae consume pulp tissues, causing premature fruit drop and rendering fruits unmarketable (Allwood et al., 2002).

The pest is further problematic because larvae complete development inside the fruit, which prevents early detection and complicates control strategies (Dong et al., 2014). After fruit drop, larvae pupate in the soil, where they undergo a prolonged diapause lasting up to 6–7 months, ensuring persistence in orchards even with seasonal variations (Liu, Qin, & Zhao, 2016). In Bhutan and other Himalayan regions, orchard sanitation practices particularly the timely collection and destruction of fallen fruits have been shown to significantly suppress subsequent population build-up (Dorji et al., 2006). Nevertheless, favorable microclimatic conditions such as mild winter temperatures, high humidity, and extended fruiting periods may exacerbate fly abundance, even in well-managed orchards (Wang et al., 2019). As a result, *B. minax* represents both a direct production threat and a trade barrier, limiting the export potential of citrus in affected regions.

Despite the recommended integrated pest management (IPM) strategies promoted by the National Plant Protection Centre, including mechanical (fruit collection and destruction), cultural (orchard sanitation), and chemical controls, farmers in Bhutan continue to face difficulties in reducing citrus fruit fly damage. Reliance on frequent insecticide applications has not yielded sustainable results, as these approaches are associated with multiple drawbacks such as pesticide residues on fruits, adverse health impacts on farmers, contamination of soil and water, disruption of natural enemies, and the risk of insecticide resistance (Victor, 2009; Wang et al., 2019). Furthermore, the absence of effective and commercially available male sex pheromone lures for *B. minax* further complicates the adoption of targeted control strategies (Dong, Zhao, & Qin, 2014).

Protein bait sprays, particularly protein hydrolysates, have been investigated as an alternative control option, but their application in tall citrus trees is often logistically challenging, limiting their effectiveness (Xia et al., 2021). Nevertheless, the biology of fruit flies makes protein-based lures promising: females require protein to support ovary development and egg production, making them highly responsive to protein-rich food sources (Vargas et al., 2010). As a result, bait stations or trap-based approaches, such as McPhail traps or modified Pet bottle traps baited with protein hydrolysates, may provide a more practical and eco-friendlier alternative for reducing fly

populations. These tools not only target females the reproductive drivers of population growth but can also be integrated with sanitation practices to enhance overall management outcomes.

In Bhutan, research on the efficacy of protein hydrolysate bait for the management of female citrus fruit flies (*Bactrocera minax*) remains scarce. Protein-based baits are a critical component of fruit fly management worldwide, as female flies require protein for ovary development and reproduction; however, their performance has not been systematically evaluated under Bhutanese citrus production systems. To address this gap, the present study was designed to assess the effectiveness of protein hydrolysate bait deployed in McPhail traps and Pet bottle traps as an alternative to female fruit flies targeted control strategies. Specifically, the study aimed to compare the relative trapping efficiency of these traps and to generate insights into the population dynamics of *B. minax* across three mandarin-growing locations in Bhutan. The outcomes are expected to inform the development of more sustainable and locally adapted integrated pest management (IPM) strategies, thereby supporting farmers to produce both long-term viability of citrus cultivation in the country and manage citrus fruit flies to increase its yield and production.

2. Materials and Method

2.1 Study Area

The study was carried out for one season in the year 2025 and was conducted in Dagana and Tsirang Dzongkhags, two of the major mandarin-producing southern regions under Agriculture Research Development Centre (ARDC), Bajo. Trapping experiments were established in three citrus orchards, each approximately 3 acres in size. Two orchards were situated in Menchuna, Drukjeygang Gewog, Dagana Dzongkhag the first orchard at 26°58'17" N, 90°00'56" E, with an elevation of 1,404 meters above sea level (masl), and the second at 26°58'31" N, 90°00'45" E, at an altitude of 1,334 masl. The third experimental site was located in Reserbu, Mendrelgang Gewog, Tsirang Dzongkhag, at 26°57'29" N, 90°07'49" E, with an elevation of 1,260 masl (figure:1). These sites were selected to represent citrus-growing areas within the altitudinal range (>1,200 masl) known to favor the emergence and population buildup of *B. minax*.



Figure 1. Map showing the study site in Drukjeygang, Dagana and Mendrelgang, Tsirang

2.2 Study Design

The trapping experiment was conducted from April to mid-August 2025 in the selected citrus orchards. Two trap types were employed, McPhail traps and modified Pet bottle traps, both baited with protein hydrolysate solution. The bait solution was prepared by dissolving 5 g of protein hydrolysate and 2 ml of malathion in 1 L of water, for field application, a standard mixture of 25 g protein hydrolysate and 10 ml malathion dissolved in 5 L of water was used. The solution was placed in all traps to attract adult fruit flies, particularly females, which require protein for ovary development and reproduction.

McPhail traps, with a cone-shaped entry funnel and a protective cover, are specifically designed to lure fruit flies into a central container that secures the bait while minimizing loss due to rain, spillage, or exposure to external conditions (figure 2 (a)). This design increases capture efficiency by reducing fly escape and maintaining bait quality. Pet bottle traps were prepared by cutting two slits measuring 3 cm × 4 cm near the upper portion of the bottle, allowing flies to enter while making escape difficult (figure 2 (b)). The bait solution was placed at the bottom of the bottle to enhance attractiveness.

At each study site, 8 McPhail traps and 8 Pet bottle traps were deployed. Trap density was maintained at approximately 5 traps per acre, ensuring sufficient spatial coverage. Traps were evenly distributed across the orchards, covering both the periphery and the interior, to account for possible variations in fly distribution. All traps were suspended at heights of 1–2 m above the ground, corresponding to the lower canopy level where fruit flies are most active. In taller trees,

traps were positioned in the lower branches to increase trapping efficiency. Because protein baits are prone to degradation under field conditions, bait solutions were monitored and replaced every two weeks to ensure consistency in fly capture.



Figure 2. McPhail Trap (a) and Pet bottle trap (b)

2.3 Data Collection

The Fruit fly counts were recorded fortnightly from April to August 2025. All flies trapped were sorted in the field, with particular emphasis on identifying and recording female *B. minax*, while male individuals were also enumerated (figure 3). Data from each trap were systematically entered into Microsoft Excel for subsequent statistical analysis. In addition, representative fruit fly specimens were collected and preserved as voucher samples for laboratory reference.



Figure 3. Sorting of *B. minax* trapped (a) in trap and female *B. minax* (b)

2.4 Data Analysis

All data analyses were carried out in RStudio version 4.4.3 (2025-02-29 ucrt). To facilitate anonymized reporting and ensure clarity, the three experimental orchards were coded as Location A, Location B, and Location C. Monitoring was conducted over seven rounds of data collection, representing fortnightly intervals from April to August 2025. Because the exact sampling dates differed across locations, observations were standardized into “Rounds” to ensure consistency in temporal comparisons.

A generalized linear mixed model (GLMM) with a negative binomial error distribution and log link function was fitted using the glmmTMB package. The fixed effects included *Trap Type* (McPhail vs Pet bottle) and *Location* (A, B, C), location A and C accounts for two orchards in Menchuna in Dagana and location B accounts for Reserbu in Tsirang respectively. While *Trap ID* and *Round* were treated as random effects to account for repeated measurements across traps and sampling intervals. This approach was chosen to appropriately model over dispersed count data and to account for hierarchical structure in the dataset.

Model coefficients were exponentiated to obtain Incidence Rate Ratios (IRR), which express the relative change in fruit fly counts compared to the reference category. An IRR greater than 1 indicated a higher fruit fly catch relative to the reference group, whereas an IRR less than 1 indicated a reduction in catch. Statistical significance of fixed effects was assessed using Wald χ^2 tests, and p-values were reported for each main effect.

This analytical framework enabled the assessment of spatial variation among orchards, trap efficiency, and temporal dynamics of citrus fruit fly populations under field conditions.

3. Result and Discussion

Each trap types, the McPhail and Pet bottle placed at different location was found effective in capturing fruit flies. However, our statistical analysis revealed a clear distinction in the effects of the variables on fruit fly catches. Interestingly, the trap type specifically, the comparison between McPhail traps and Pet Bottle traps was found to have no statistically significant effect on the number of fruit flies captured (IRR = 0.77, $p = 0.298$) shown in Table 1. This suggests that both trap types performed similarly in monitoring the citrus fruit fly population.

Table 1. Showing the overall effects of trap type and location on fruit fly catches from the GLMM analysis.

Factor	Level/Comparison			Estimate (log scale)	IRR (exp β)	SE	p-value
Trap type	McPhil bottles	vs	Pet	-0.264	0.77	0.254	0.298
Location	B vs A			1.269	3.56	0.331	0.001
Location	C vs A			0.925	2.52	0.331	0.005

In contrast, the trap location had a profound and significant impact on the results. Traps placed at Location B showed a substantial increase in fruit fly catches compared to Location A (IRR = 3.56, $p < 0.001$). Similarly, traps at Location C also yielded significantly higher counts than those at Location A (IRR = 2.52, $p = 0.005$). These findings strongly indicate that while the choice of trap type didn't influence the number of fruit fly catches, the specific location of the trap was a crucial determinant of its effectiveness.

The most significant finding of this study is the comparable performance of the McPhail and Pet bottle traps. The lack of a significant difference between these two trap types demonstrates that both are similarly effective for monitoring/managing citrus fruit flies. This has important implications for integrated pest management (IPM) strategies, particularly in contexts like Bhutan, where cost is a major consideration for farmers.

As per the National Plant Protection Centre's (NPPC) price list for the fiscal year 2025-2026, a single McPhail trap costs Ngultrum 200. For large-scale monitoring in bigger orchards, deploying a large number of these traps could become very expensive. The fact that the much more accessible and cost-effective Pet Bottle traps are just as effective provides a viable and sustainable alternative. Farmers can easily acquire and deploy these traps, which could greatly enhance the feasibility of large-scale fruit fly managing programs, ultimately supporting effective pest control efforts throughout the region.

Interestingly, despite Location C exhibiting relatively good orchard sanitation and management practices, fruit fly counts remained high. This suggests that favorable environmental conditions, such as temperature, humidity, and rainfall patterns, may have supported fruit fly activity and survival independent of sanitation levels. Microclimatic influences of this kind have been highlighted in several studies. For example, Fu et al. (2023) found that *Bactrocera minax* adult activity is maximized under moderate temperatures (~20-23 °C), and declines sharply when temperatures exceed ~30 °C, showing that deviations in local temperature significantly affect survival and behavior.

Similarly, a recent survey of *Bactrocera* species in mango orchards of Uttar Pradesh demonstrated that minimum temperature, relative humidity, and rainfall were strongly correlated with population peaks during monsoon/post-monsoon periods. In addition, a study by Zhang et al., 2025 suggest that microclimatic effects of cooler temperature and different humidity play a significant role in emergence timing and mortality of immature stages finally, Sharma & Dahal (2020) observed that in mid-hill regions of Nepal, moderate temperature combined with lower humidity favored rapid population increase of *B. minax*, whereas overly humid conditions reduced survival and reproduction.

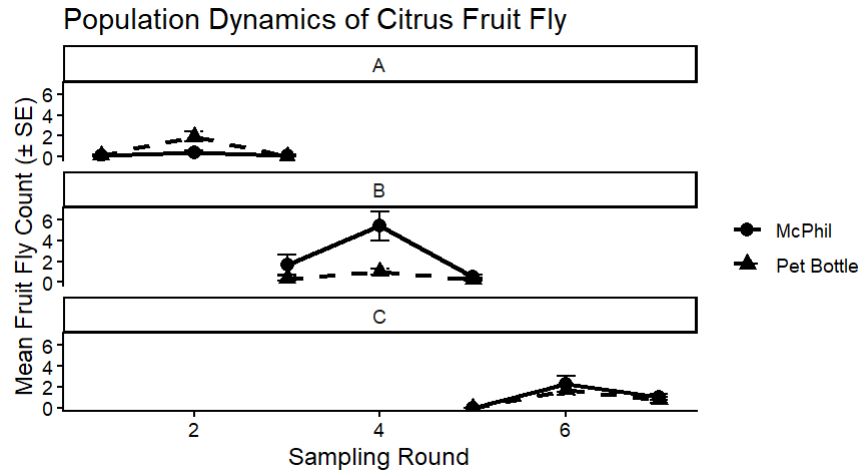


Figure 4. Population dynamics of citrus fruit fly across sampling rounds in three locations (A–C) using McPhail and Pet Bottle traps. Error bars represent standard errors of the mean ($\pm$ SE).

The population dynamics of citrus fruit fly varied across locations and trap types. In Location A, fruit fly catches remained relatively low throughout the study, with a slight increase at Round 2 followed by a decline. In contrast, Location B recorded the highest fly abundance, peaking sharply at Round 4, particularly in McPhail traps shown in figure 4. Location C showed overall low catches but displayed a modest peak during Round 6. Across all locations, McPhil traps consistently captured more flies than Pet Bottle traps, however statistical analysis showed no significant differences between the two trap types.

The observed trends suggest that fruit fly abundance is influenced by both local conditions and trap type. The pronounced peak in Location B may be attributed to poor orchard sanitation, as uncollected fruit drops provide breeding sites for fruit flies. Similar findings have been reported by Dorji et al. (2007), who highlighted the importance of fruit collection in reducing subsequent fly emergence. Conversely, despite relatively good sanitation practices in Location C, fly catches increased at Round 6, possibly due to favorable microclimatic conditions such as temperature and humidity, which are known to enhance fruit fly survival and activity (Xia et al., 2021). These results underscore that while sanitation is critical, broader environmental factors must also be considered in integrated management.

4. Conclusion

This study provides new insights into the management and population dynamics of the citrus fruit fly, a major pest threatening mandarin orange production in Bhutan. By evaluating the effectiveness of protein hydrolysate bait in McPhail and Pet bottle traps across three citrus-growing orchards, we demonstrated that trapping efficiency varied both by trap type and location, reflecting the influence of orchard sanitation, local management practices, and microclimatic conditions. While McPhail traps showed relatively higher fruit fly catch compared to Pet bottle traps, the consistent captures across both trap types confirm the potential of protein bait as a practical alternative for monitoring and reducing female fruit fly populations.

The study also revealed strong spatial variation, with significantly higher fruit fly counts recorded in sites with poor orchard sanitation, reinforcing previous findings that proper field hygiene plays a critical role in fruit fly suppression. Nevertheless, the high catches observed in Location C, despite better sanitation, highlight the importance of environmental drivers such as temperature, humidity, and rainfall in shaping fruit fly population dynamics. These results emphasize that fruit fly management cannot rely on a single strategy, but requires an integrated approach that combines orchard sanitation, protein bait trapping, and context-specific cultural practices.

Overall, this research addresses a key gap in Bhutanese fruit fly studies by generating location-specific evidence on trap performance and population trends of *B. minax*. The findings provide a baseline for the development of locally tailored integrated pest management (IPM) strategies that balance effectiveness, farmer feasibility, and sustainability. Further research should focus on the role of climate change in population dynamic of fruit flies, assessing long-term impacts of sanitation practices under varying ecological conditions. Strengthening research and extension support in this field will be critical for safeguarding Bhutan's mandarin orange sector, which remains both a vital export commodity and a cornerstone of rural livelihoods.

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6. Authors' contribution statement

- Study conception and design: Ugyen Dema and Thinley Wangmo
- Implementation of research and data collection: Ugyen Dema and Thinley Wangmo
- Analysis and interpretation of results: Ugyen Dema and Thinley Wangmo
- Draft manuscript preparation: Ugyen Dema and Thinley Wangmo.

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