Emerging Trends in Fruit Fly Diagnostics and its Sustainable Management

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Fruit flies are notorious pests of agri-horticultural crops and represent one of the major biotic stresses responsible for reducing economic yield and market value. They are highly polyphagous and feed on both flowers and fruits. Since the larval stages remain concealed inside fruits, it is difficult to identify infested fruits and vegetables, resulting in substantial economic losses. Precise identification of fruit flies at immature stages is almost impossible, while identification at the adult stage is also challenging due to variations in coloration patterns, the presence of cryptic species, and sexual dimorphism, necessitating the involvement of expert taxonomists. Advancements in molecular techniques, such as DNA barcoding, have become important tools for identifying fruit fly species, although certain limitations still exist. Species-specific markers, quantitative PCR (qPCR), multiplex PCR, and isothermal amplification assays have emerged as valuable techniques for species-specific diagnostics. These methods improve the accuracy and speed of identification while reducing diagnostic costs. The management of fruit flies largely depends on male-specific attractants and pheromones, whereas effective female-attractive pheromones are still not available. Pre-harvest fruit bagging has emerged as a cost-effective technique for high-value fruit crops and has been found to reduce fruit fly incidence and damage while providing additional benefits. Post-harvest management approaches, including hot-water treatment, vapour heat treatment, and irradiation, continue to be employed for export commodities to eliminate different developmental stages of fruit flies present in fruits.

Keywords: *Fruit flies, Pest, DNA barcoding, PCR, Post-harvest treatment, fruit bagging*

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Introduction

Fruit flies represent one of the most economically destructive groups of insect pests affecting global horticulture, owing to their wide host range, high reproductive capacity, and severe impact on fruit quality and marketability (Badii et al., 2015). Fruit flies belonging to the genus *Bactrocera*, *Zeugodacus*, *Ceratitis*, *Dacus* and *Rhagoletis* are major pests and are responsible for major pre and post-harvest losses in fruits and vegetables across the world (Clarke, 2019). Fruit flies belong to the family Tephritidae and the order Diptera. More than 5000 species of fruit flies belong to the Tephritidae are described in the world and among them 200-250 species are considered as pests. The yield losses caused by them in the range of 30-40% but it may go up to 80-90% in case of severe damage. The direct loss is caused by the female by oviposition and hatched maggots feed on the fruits and vegetables make them unfit for human consumption and marketing. The infested fruits drop prematurely, causing internal rotting, untimely fruit drop leading to yield reduction and it may go up to 80% in non-managed field (Opoku et al., 2025).

Beyond direct crop damage, fruit flies impose significant indirect economic costs through quarantine restrictions and trade barriers. Many species of fruit flies are categorized and considered as quarantine pests, and their presence in the production areas entails costly compliance guidelines, such as, post-harvest treatments, certification protocols, and area-wide pest management programs (Hoskins et al., 2023). Horticulture system that is export-oriented suffers excessively, as the importing countries has strict enforcement policies for fruit fly infestation. That will result in shipment rejections, trade barriers, and declining export revenues (Sarwar, 2015), and the financial burden will further become more severe that will be amplified by total cost associated with surveillance, monitoring, and control, that will include the use of insecticides, bait sprays, pheromone-based trapping systems, and sterile insect technique (SIT) programs.

Low-income farmers face a severe crop loss from fruit fly infestations and surging an extra expenditure on pest management strategies that threaten farmer’s profit and financial security (Mugure, 2012). In numerous developing countries, frequent application of pesticides for fruit fly infestation control can increase expenses and endangers for human health, native habitats, and environmental sustainability. Additionally, the presence of pesticide residue can further compromise market acceptance, and creating another financial hurdle (Verdadero et al., 2025). At a national and regional scale, fruit fly outbreaks can disrupt entire value chains, affecting producers, traders, processors, and exporters alike (Musasa et al., 2017). Overall, the economics of fruit flies extend well beyond field-level yield losses, encompassing trade limitations, increased production and compliance costs, and long-term impacts on market stability. Consequently, investment in integrated, biologically informed, and area-wide fruit fly management strategies is economically justified and essential for sustaining profitable and competitive horticultural production systems.

Biology of fruit flies

Fruit flies which are economically important and infest agri-horticultural crops worldwide belongs to the family Tephritidae and order Diptera. They are of major concern because of polyphagy, high reproductive potential, and destructive larval feeding habits (He et al., 2023). The Prominent fruit fly species in India (Table.1) such as Oriental fruit fly, *Bactrocera dorsalis*, Peach Fruit Fly (*Bactrocera zonata),* Guava Fruit Fly *(Bactrocera correcta),* Chinese Citrus Fly *(Bactrocera minax),* Melon Fruit Fly *(Zeugodacus cucurbitae)* Pumpkin fruit fly (*Z.tau),* whereas Queensland Fruit Fly (*Bactrocera tryoni*) in Australasian region, Apple Maggot Fly (*Rhagoletis pomonella*) in USA, and Mediterranean Fruit Fly / Medfly (*Ceratitis capitata*) in South Africa is a major and prominent species respectively and they are holometabolous in nature having egg, larva/maggot, pupa and adult stages. Females have well developed ovipositor, which enables them to insert a group of eggs beneath the skin/epidermis of under/half/ripened fruit. Eggs hatch within 1-3 days, releasing apodous maggots that feed voraciously on the fruit pulp, causing internal breakdown, premature fruit drop, and secondary microbial infections which hastens further fruits deterioration (Ghodekar et al., 2025). The larval stage typically consists of three instars and completes in 3 to 8 days under normal weather, which also represents the most damaging phase of the life cycle. After complete larval development, mature larvae/maggots drop in to the soil for pupation, where metamorphosis occurs. The total pupal phase varies with the environmental temperature and moisture of the soil and typically ranges from 7 to 15 days under tropical conditions (He et al., 2023).

Fruit fly adults are long-lived and typically survive for a few weeks to up to months depending upon food availability and climatic conditions (Carey & Molleman, 2010). Adults require food that is rich in carbohydrates for their survival and proteinaceous diets for sexual maturation and fecundity (Momen et al., 2025). Males of several *Bactrocera* and *Zeugodacus* species respond strongly to male attractants such as methyl eugenol and cue-lure, respectively, a behavioral trait exploited in monitoring and management programs (Shelly, 2010). Fruit flies show higher dispersal ability and overlapping generations, which will allow a rapid increase in population during favorable seasons. Their biology is influenced by climatic factors, host availability, and orchard management practices (Aluja et al., 2012). The intimate association of larval development with marketable fruit tissues, coupled with cryptic infestation, makes fruit flies particularly difficult to manage and emphasizes the necessity of understanding their biology for designing effective, sustainable control strategies.

Figure 1. Field observations and monitoring of tephritid fruit flies

(A) Maggots of fruit flies feeding within infested guava fruit. (B) Fruit damage caused by larval infestation in mango. (C) Bottle trap equipped with a para-pheromone lure for field monitoring of adult fruit flies. (D) Adult tephritid fruit flies collected in the baited trap

Quarantine importance of fruit flies

Fruit flies are the major quarantine pests worldwide because of their highly invasive nature, broad host range, and economic importance in horticultural trade and food security (Sarwar, 2015). Many countries designated various fruit fly species as quarantine pests due to extensive damage they inflict on fruits and vegetables and their ability to establish rapidly in new environments (Hendrichs, 2020). The quarantine importance of fruit flies stems from their biology and behavior: females oviposit eggs inside healthy fruits, where larvae feed and develop, rendering produce unmarketable and facilitating hidden transport across borders. International movement of fresh produce, planting material, and even passenger baggage significantly increases the risk of accidental introduction of these pests into fruit-fly-free or low-prevalence areas. After establishment in a particular region, it will be very difficult and costly to eradicate fruit flies due to their high reproductive rates, multivoltine life cycles, and easy adaptability to diverse climatic conditions (Niazee & Taskeen, 1988). From an international trade perspective, the detection of even a single quarantine fruit fly species is sufficient to immediately trigger export bans, implementation of phytosanitary restrictions, and loss of market access, leading to substantial economic losses for producing countries (Arya et al., 2024).

In 2021, the European Food Safety Authority (EFSA) compiled and developed a pest survey card for *B. zonata* at the request of the European Commission to facilitate standardized and efficient data recording by EU Member States on the pest's status. This tool helps in evidence-based risk assessment and the formulation of sound management strategies, particularly in response to the recent introduction of the pest through infested fruits of *Prunus* species. Similar survey cards have been previously developed for *B. dorsalis* (EFSA, 2019), *R. pomonella* (EFSA, 2020a), and *R. fausta* (EFSA, 2020b). As a result, national plant protection organizations implemented rigorous quarantine measures, including pre- and post-harvest treatments, pest-free area certification, surveillance using attractant-based traps, and strict inspection and disinfestation protocols at ports of entry. International frameworks such as the International Plant Protection Convention and guidelines developed under the Food and Agriculture Organization emphasize harmonized phytosanitary standards to prevent transboundary movement of fruit flies while facilitating safe international trade (IPPC, 2018). Effective quarantine management not only protects domestic agriculture and biodiversity from invasive fruit fly incursions but also supports sustainable international trade by ensuring compliance with importing country regulations. Therefore, fruit flies remain a major focus in plant quarantine systems worldwide, and continue to highlight the need for strengthening their surveillance, risk analysis, early detection, and exclusion strategies that is essential to mitigate their global threat.

Table 1. Some economically important fruit fly species from India.

Fruit fly species	Host preferences	Major crop groups infested	Reference
Bactrocera. dorsalis	Highly polyphagous	Mango, guava, citrus, banana, and papaya	Ahmad et al. (2024)
B. correcta	Moderately polyphagous	Guava, mango, and peach	Ahmad et al. (2023)
B. zonata	Polyphagous	Mango, guava, peach, and citrus	Sarwar et al. (2013)
B. minax	Oligophagous	Citrus fruits and (Rutaceae) Restricted to Sikkim and West Bengal	Ghazmer et al., 2023
Z. cucurbitae	Oligophagous	Cucurbits	Arya et al. (2026)
Z. tau	Oligophagous	Cucurbits	Boopathi et al. (2017)

B. divenderi	Narrow host range	Peach and Nectarine	Prabhakar (2022)
Z. diversus	Narrow host range	Cucurbits	David et al. (2024)
Z. caudatus	Oligophagous	Cucurbits	Laskar et al. (2016)
Carpomya vesuviana	Monophagous	Ber	Kavin et al. (2024)

Recent advances in the molecular diagnostics of fruit flies of economic importance

Fruit flies are traditionally identified based on adult morphological characters such as wing colouration, wing venation, position of setae, ocelli position, body colouration, and male and female genitalia (Chandrashekhar et al. 2024; Hatwar et al. 2025a, 2026b). However, these characters are often difficult to interpret by non-taxonomists, and even experts may encounter challenges that lead to misidentification. Moreover, morphological identification is largely restricted to the adult stage, as eggs, maggots, and pupae lack distinct taxonomic characters and are therefore almost impossible to identify reliably. Advances in molecular biology have greatly simplified diagnostic procedures through various molecular approaches, which can be effectively applied even by non-taxonomists for accurate species identification. Correct and timely identification of fruit flies is of paramount importance for implementing species-specific management strategies (such as the application of para pheromones and eradication programmes), for providing import permits for fruits, vegetables, and other consignments to prevent the introduction of exotic species into the country, and for facilitating the export of agricultural produce to other countries. Molecular approaches for the identification of fruit flies include DNA barcoding, species-specific primers, isothermal amplification assays, and genome-editing-based tools. These approaches have simplified what were previously complex identification processes, and many of these techniques can be effectively applied in basic laboratory setups, for field-level detection, as well as at quarantine stations.

Molecular identification of fruit flies

a. Mitochondrial cytochrome oxidase I (mtCOI)-based DNA barcoding remains one of the most widely applied molecular tools for species identification in economically important fruit flies (Diptera: Tephritidae) (Arya et al. 2022; Chandrashekhar et al., 2024) as it can distinguish species across different life stages and is effective irrespective of sex. However, its utility can be limited by the quality of reference databases, lack of representation for all species, potential misidentifications in public sequence repositories, and insufficient genetic divergence among closely related species, making accurate diagnosis challenging without high-quality sequencing and robust comparative data (Chonticha & Pairot 2019). Its applications also limited in the cryptic species such as *Anstrepha fraterculus* species complex (Bartolini et al., 2020.)

b. Species-specific primer-based identification

The Species-specific primers (SSPSs) are those primers which identify the target species by producing amplification while no amplification in the non-target species and no template control at a particular annealing temperature. The SSPs based molecular assays have emerged as reliable tools for the rapid and accurate identification of economically important fruit flies. These primers are designed to amplify unique regions of mitochondrial or nuclear genes (such as COI, ITS, or 16S rRNA) that are diagnostic for a particular species or species complex. This approach allows unambiguous identification without the need for sequencing, making it faster and more cost-effective than DNA barcoding. Species-specific PCR assays are effective across all developmental stages, including eggs, larvae, pupae, and adult and are particularly valuable for quarantine and biosecurity applications. However, their utility is limited to known target species, and careful primer validation is essential to avoid cross-amplification among closely related taxa. Species specific primers for oriental fruit fly, *Bactrocera dorsalis* (Arya et al. 2024), and for *Bactrocera correcta* (Jiang et al., 2023) has been developed. The multiplex PCR is an advanced concept where multiple species of fruit flies are identified in the one PCR reaction, and it's a valuable to for identification of multiple species at one time. Andrews et al. 2022 developed multiplex PCR assay for the detection of fruit flies of economic importance to South Africa.

c. Quantitative real-time PCR (qPCR)-based species-specific identification

The qPCR can detect small quantity of target DNA with high accuracy and no need for Gel electrophoresis to confirm the results. Due to real-time monitoring of data the fluorescence-based detection enables quick species identification and even DNA concentration. These systems are best fit for quarantine stations for detection of fruit flies accurately. In qPCR, both mitochondrial and nuclear based species-specific primers can be used and even multiflexed. "A species-specific real-time PCR assay targeting the mitochondrial cytochrome oxidase I (COI) gene was developed for rapid identification of the melon fruit fly *Zeugodacus cucurbitae* (Diptera: Tephritidae) from other closely related fruit fly species (Arya et al., 2026). This assay demonstrated high specificity -with no cross-reaction against non-target tephritid species and high sensitivity, enabling rapid and precise species discrimination irrespective of developmental stage, sex, or specimen condition. The method is suitable for routine diagnostics and quarantine screening to support phytosanitary decisions and biosecurity surveillance (Kayattukandy et al., 2025; Arya et al., 2026). Similarly, the qPCR-based assay for detection has been developed for the following the *Bactrocera philippinensis* and *B. occipitalis* (Yu et al. 2005)

d. Field level molecular detection

Isothermal amplification assays (IAAs) are molecular techniques used to amplify nucleic acids (DNA or RNA) at a constant temperature, eliminating the need for a thermal cycler, gel electrophoresis and sequencing. Because they are rapid, sensitive, and equipment-light, they are especially useful for field diagnostics, quarantine stations, and low-resource laboratories. The most widely used isothermal amplification assays (IAAs) in plant protection are Loop-Mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA). These methods operate at constant temperatures of 60-65 °C and 37-42 °C, respectively. Although both assays rely on isothermal amplification, they differ in their underlying principles and in the number and design of primers used.

The end-point results can be visualized either by colorimetric changes or by agarose gel electrophoresis; however, with prior assay standardization, the electrophoresis step can be omitted, thereby significantly reducing detection time. Both methods require the design of highly specific primers, and optimization of primer concentration in the reaction master mix plays a pivotal role in achieving accurate and reliable species-specific detection. LAMP-based diagnostic assays have been developed for several economically important fruit fly species, including the melon fruit fly (*Zeugodacus cucurbitae*) (Barman et al., 2025), the Queensland fruit fly (*Bactrocera tryoni*) (Blacket et al., 2020), and the New Guinea fruit fly (*B. trivialis*) (Starkie et al., 2022). Similarly, RPA-based diagnostics integrated with CRISPR/Cas systems have been reported, such as an RPA-CRISPR/Cas12b assay for the oriental fruit fly (*B. dorsalis*) (Lv et al., 2025) and an RPA-CRISPR/Cas12a assay for the guava fruit fly (*B. correcta*) (Li et al., 2024).

Recent advances in the management of fruit flies of economic importance

a. Behaviour control: Behavioral Control Using Parapheromones and Food Baits

Parapheromones constitute an important component of behavioral control strategies for fruit fly management. Species-specific parapheromones are widely used in attract-and-kill approaches, wherein adult fruit flies are attracted and subsequently eliminated using insecticides, drowning mechanisms, or sticky traps (Sunda et al., 2024). This strategy is practiced worldwide and is particularly effective because parapheromones predominantly attract male fruit flies, thereby reducing mating success with females. The consequent reduction in fertilization leads to suppression of subsequent generations. Parapheromones are highly volatile compounds capable of attracting fruit flies from long distances, sometimes extending up to several kilometers. In India, the most widely used parapheromones are methyl eugenol (ME) [benzene, 1,2-dimethoxy-4-(2-propenyl)] and cue-lure [4-(4-acetoxyphenyl)-2-butanone], which are effective against fruit- and vegetable-infesting fruit flies, respectively (Arya et al., 2022). Several studies have evaluated the effect of lure concentration on trapping efficiency, and although higher concentrations generally result in increased trap catches, the most effective and economically viable formulation has been reported as 6:4:2 or 6:4:1 (ethanol: pheromone lure: insecticide) (Arya et al., 2025). Methyl eugenol occurs naturally in holy basil (*Ocimum tenuiflorum*), particularly in the chemotype DOS-1, where its concentration can reach approximately 75% (Kothari et al., 2004; Saran and Meena, 2018). This plant can be exploited either for extraction of methyl eugenol or for fruit fly management through intercropping or trap preparation using discarded basil biomass.

Recent advancements in behavioral control include the development of nano-lures, which enhance the controlled and prolonged release of parapheromones throughout the cropping period, thereby improving trap longevity and efficacy (Bhagat et al., 2013). In addition to parapheromones, protein-based food baits play a crucial role in fruit fly management. Adult females require protein for sexual maturation and egg development, while both sexes depend on protein for survival. Protein baits, commonly formulated with yeast or protein hydrolysates, release ammonia-based volatiles that mimic natural food sources, making them particularly attractive to protein-starved females. This characteristic renders protein baits highly effective for population suppression. Based on this principle, dual-sex attractive food baits have been demonstrated to be effective against the melon fruit fly, *Zeugodacus cucurbitae* (McCracken et al., 2023).

b. Pre-Harvest Fruit bagging

Generally, fruits and vegetables are attacked by wide variety of insect pests and diseases, and their management relied on insecticides and pesticides. Due to non-scientific application such as over dose, spurious pesticides, banned pesticides leads to negative consequences such as rejection of consignments by importing countries due to non-compliance with pesticide maximum residue limits (MRLs). To minimize such risks pre-harvest fruit bagging has become a Good Agricultural Practice (GAP) especially in high valued crops like mango, guava, apple, peach, dragon fruit, banana and others. Pre-harvest fruit bagging is at right time has been reported to confirm multiple benefits, including the reduced infestation of insect pests and diseases, lower or negligible pesticide residues, improved physical appearance and bio-chemical qualities of the fruits. The bagged fruits also exhibit improved length and weight, free from surface blemishes, sunburn and bird damage along with increased content of total soluble solids (TSS) and ascorbic acid. These fruits fetch better market price and high potential for export (Sharma et al., 2014; Xia et al., 2019). Despite the well-documented qualitative advantages, information on the cost-benefit ratio of fruit bagging under different production systems remains limited and requires systematic evaluation.

The effectiveness of fruit bagging is strongly influenced by factors such as type and quality of bagging material, timing of bag application, and post-harvest handling practices (Sharma et al., 2014; Xia et al., 2019; Srivastava et al., 2023). Recognizing its importance, many Indian states (Andhra Pradesh, Telangana, Uttar Pradesh, Bihar etc.) are currently promoting pre-harvest paper bagging through subsidy schemes, encouraging adoption among fruit growers for sustainable and residue-free production.

c. Post-harvest management

Many countries enforce strict quarantine measures before issuing import permits for fruits and vegetables to prevent the introduction of invasive fruit fly species. The most widely recommended measures include sourcing fruits from designated pest-free areas, hot water treatment (Jacobi et al., 2001), vapour heat treatment and irradiation to eliminate different developmental stages of fruit flies. By 2011, only two thermal disinfestation facilities were registered in India; however, recognizing their importance in promoting pest-free fruit exports, this number has increased to 52 facilities registered with the Directorate of Plant Protection, Quarantine and Storage (DPPQS). Despite this progress, these facilities are largely confined to Maharashtra, Gujarat, and a few southern states, and there is an urgent need to extend such infrastructure to other regions of the country to enhance pest-free exports (Verghese et al., 2024). In addition, non-destructive detection methods such as thermal imaging, X-ray, and infrared-based technologies are emerging for the detection and sorting of fruit fly-infested fruits and vegetables (Sobha et al., 2024; Lu & Ariana, 2013). These technologies show considerable promise once practically viable products are developed for large-scale application. Notably, a start-up company, Wide Mobility, has developed an X-ray-based detection system (Conrad-G) for identifying fruit fly infestation (melon fly and *Dacus* spp.) in gherkins. This technology has significantly reduced consignment rejections and helped exporters meet global food export standards.

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