UNIVERSIDADE
EDUARDO
MONDLANE

FACULTY OF AGRONOMY AND FORESTRY ENGENEERING

DEPARTMENT OF PLANT PROTECTION

MASTER COURSE IN PLANT PROTECTION

EFFECTIVENESS OF FRUIT FLIES MANAGEMENT STRATEGIES WITH EMPHASIS ON ESTABLISHMENT AND DISPERSAL OF PARASITOIDS IN MOZAMBIQUE

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Maputo, June 2026

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A Dissertation Submitted to the Eduardo Mondlane University - Department of Plant Protection, under the supervision of Prof. Doutor Domingos Cugala and Doutora Laura José Canhanga, Engª , in Fulfillment of the Requirements for Master Degree in Plant Protection.

Maputo, June 2026

DECLARATION

"I declare that this dissertation has never been submitted for the purpose of obtaining any degree or in any other field and that it is the result of my individual labor. This dissertation is presented in partial fulfillment of the requirements for obtaining the degree of master in plant protection, from Eduardo Mondlane University".

GONZARVE NTAWIZERA

……Date…….

I confirm that the work reported in this dissertation was carried out under my supervision.

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ABSTRACT

Fruit flies (Diptera: Tephritidae) are among the most destructive pests affecting fruit production worldwide and in Africa especially *B. dorsalis* causing significant economic losses and restricting horticultural trade. In Mozambique, this species attacks a wide range of hosts, with potential yield losses reaching up to 80% in the absence of effective management. This study evaluated the effectiveness of integrated fruit fly management strategies in Mozambique, with emphasis on bait stations, male annihilation technique (MAT), host infestation levels, and biological control using parasitoids. Field experiments were conducted in Boane District using three treatments: control, M3 bait stations alone, and M3 combined with MAT. Fruit fly populations were monitored using torula-baited traps, while infestation indices were determined through fruit collection and incubation. In Cabo Delgado Province, host fruits were sampled at varying distances from parasitoid release sites to assess parasitism and dispersal. A total of 3450 fruit flies belonging to *Bactrocera*, *Dacus*, and *Ceratitis* were captured, with *B. dorsalis* being the dominant species (64–68% of total captures), followed by *D. bivittatus*. Fruit fly density of *B. dorsalis* significantly decreased from 8.68 flies/trap/day in the control to 4.07 and 1.25 in the M3 and M3+MAT treatments, respectively, indicating strong suppression effects. Similar trends were observed for *D. bivittatus*. Infestation levels varied significantly among host fruits, with guava showing the highest indices, followed by tropical almond, while citrus and june plum exhibited the lowest infestation levels. Mango showed moderate infestation across parameters. Parasitoid surveys recorded *D. longicaudata* and *P. cosyrae* in Cabo Delgado, while *F. arisanus* was not recovered in any location. In Maputo, only *P. cosyrae* was detected. In Cabo Delgado province, parasitism rates varied across sampling locations, ranging from 3.16% to 10.93%. *P. cosyrae* was the most widely distributed parasitoid species and was detected in all sampled sites. In contrast, *D. longicaudata* was recorded only in selected locations, where it exhibited higher parasitism rates than *P. cosyrae*, particularly in Mieze (6.55%) and Komakoma (5.5%). Despite relatively high host sample numbers in some areas, parasitism levels remained low to moderate across the study sites, indicating spatial variation in parasitoid effectiveness. The maximum dispersal distance reached 74.5 km. The observed parasitism and dispersal rates of *D. longicaudata* suggests that future management strategies should prioritize the conservation and augmentation of parasitoids in Cabo Delgado and Maputo Provinces.

Keywords: Fruit flies; *D.longicaudata*; dispersal; M3 bait stations; male annihilation technique.

RESUMO

As moscas das frutas (Diptera: Tephritidae) estão entre as pragas mais destrutivas que afectam a produção de frutas em todo o mundo e na África, especialmente a *B. dorsalis*, causando perdas econômicas significativas e restringindo o comércio de produtos hortícolas. Em Moçambique, essa espécie ataca uma ampla gama de hospedeiros, com perdas de produtividade chegando a 80% na ausência de um maneio eficaz. Este estudo avaliou a eficácia de estratégias de maneio integrado de moscas das frutas em Moçambique, com ênfase em estações de ísca, na técnica de aniquilação de machos (MAT), e no controle biológico utilizando parasitoides. Ensaios foram realizados no distrito de Boane utilizando três tratamentos: controle, estações de ísca M3 isoladas e M3 combinada com MAT. As populações de moscas das frutas foram monitoradas com armadilhas contendo Torula, enquanto que os índices de infestação foram determinados por meio da colecta e incubação de frutos. Na província de Cabo Delgado, frutos hospedeiros foram amostrados a distâncias variadas dos locais de liberação de parasitoides para avaliar o parasitismo e a dispersão. Um total de 3450 moscas das frutas pertencentes aos gêneros Bactrocera, Dacus e Ceratitis foi capturado, sendo a *B. dorsalis* a espécie dominante (64–68% do total de capturas), seguida pela *D. bivittatus*. A densidade populacional de *B. dorsalis* diminuiu significativamente de 8.68 moscas/armadilha/dia no controle para 4.07 e 1.25 nos tratamentos M3 e M3+MAT, respectivamente, indicando fortes efeitos de supressão. Tendências semelhantes foram observadas para *D. bivittatus*. Os níveis de infestação variaram significativamente entre os frutos hospedeiros; tendo a goiaba apresentado os índices mais elevados e a manga apresentou infestação moderada. Levantamentos de parasitoides registraram *D. longicaudata* e *P. cosyrae* em Cabo Delgado, enquanto *F. arisanus* não foi recuperada em nenhum local. Em Maputo, apenas *P. cosyrae* foi detectada. Na província de Cabo Delgado, as taxas de parasitismo variaram entre os locais de amostragem, oscilando entre 3.16% e 10.93%. *P. cosyrae* foi a espécie de parasitoide com maior distribuição, sendo detectada em todos os locais amostrados. Em contrapartida, *D. longicaudata* foi registrada apenas em alguns locais, onde apresentou taxas de parasitismo mais elevadas do que *P. cosyrae*, particularmente em Mieze (6,55%) e Komakoma (5,15%). A distancia máxima de dispersão atingiu 74.5 km. O parasitismo permaneceu baixo a moderado, sugerindo que as futuras estratégias de maneio devem priorizar a conservação e o incremento das populações de parasitoides nas províncias de Cabo Delgado e Maputo.

Palavras-chave: Moscas-das-frutas*; D. longicaudata*; dispersão; estações de isca M3; técnica de aniquilação de machos.

DEDICATION

I dedicate this work to my beloved parents, Ignace Kagiraneza and Annonciata Nyirampeta, for their moral support, encouragement, and sacrifices throughout my academic journey. I also dedicate this work to my dear siblings for their constant support and motivation.

ACKNOWLEDGEMENTS

This dissertation marks an important milestone in my academic journey, and I would like to acknowledge the individuals and institutions that contributed to its completion.

I am grateful to the Centre of Excellence in Agrifood Systems and Nutrition (CE-AFSN), under World Bank for awarding me scholarship and research funding to facilitate my studies and this research work.

I would also like to extend my sincere appreciation to Eduardo Mondlane University, particularly the Faculty of Agronomy and Forestry Engineering and the Department of Plant Protection, for the opportunity to pursue my studies. I am grateful for the quality training, research facilities, and supportive academic environment that greatly contributed to my academic growth and the successful completion of this work.

My sincere appreciation goes to my supervisors, Prof. Doutor Domingos Cugala and Doutora Laura José Canhanga, for their continuous support, professional guidance, and critical insights. Their availability, constructive comments, and commitment to my progress were essential throughout the entire research process

Special thanks are extended to all individuals especially my classmate who contributed during fieldwork and data collection. Their cooperation and assistance were highly appreciated.

I express my deep gratitude to my family and friends for their patience, encouragement, and unwavering support during this demanding period.

I am deeply grateful to the Almighty God for His abundant grace and guidance, which enabled me to successfully complete this work. May His name be praised forever.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDECES

ABBREVIATIONS AND ACRONYMS

ANOVA : Analysis of Variance

CABI : Centre for Agriculture and Bioscience International

CIP : Crop Improvement Program

CIMMYT : International Maize and Wheat Improvement Center

DAE : Department of Agricultural Engineering

DDVP : Dichlorovinyl dimethyl phosphate

EC : Emulsifiable Concentrate

EPPO : European and Mediterranean Plant Protection Organization

FAO : Food and Agriculture Organization

GBIF : Global Biodiversity Information Facility

GDP : Gross Domestic Product

GIS : Geographic Information System

ICIPE : International Centre of Insect Physiology and Ecology

IPM : Integrated Pest Management

IRRI : International Rice Research Institute

MAT : Male Annihilation Technique

MRR : Marginal Rate of Return

NARS : National Agricultural Research Systems

NGO : Non-Governmental Organization

PPE : Personal Protective Equipment

R&D : Research and Development

SADC : Southern African Development Community

1.0 INTRODUCTION

1.1. Background

Agriculture is one of the most important sectors globally, contributing significantly to economic growth, food security, and poverty reduction, particularly in developing countries. It accounts for approximately 5% of the global Gross Domestic Product (GDP) and provides employment for a large proportion of the population, especially in rural areas. The sector also plays a vital role in national economies through both domestic production and export markets. In 2022, global fruit and vegetable production reached approximately 1.9 billion metric tons, with developing countries contributing over 80% of this output, highlighting the importance of horticultural crops in supporting livelihoods and rural incomes worldwide (FAO, 2023).

In Mozambique, agriculture remains a cornerstone of the economy, contributing about 26.3% of the GDP and employing more than 70% of the population. Favorable agro-climatic conditions further enhance Mozambique's potential for agricultural production and export. In addition, government initiatives aimed at agricultural diversification, together with private sector involvement, have stimulated the expansion of fresh fruit production and commercialization. The country produces approximately 0.7 to 1 million tons of fruits annually, reflecting the sector's importance for both subsistence and commercial purposes (Mole & Popat, 2024; Muyambiri, 2023). Despite this potential, fruit production in Mozambique faces several constraints, among which fruit flies are one of the most significant. These pests cause premature fruit drop, reduce fruit quality, and limit market access for both fresh consumption and industrial use (Canhanga et al., 2021).

Fruit flies can cause significant financial losses to fruits, making effective control measures essential (Papadopoulos et al., 2024). In Mozambique, losses in mango orchards exceed USD 3,400 per hectare and may be even higher in other cropping systems where growers often do not implement control measures. There are several control strategies recommended for African context, that can cost to growers approximately USD 1,000 to USD 2,000 per hectare annually due to pesticide applications (Bota et al., 2020).

In response to these challenges, integrated pest management strategies have been promoted to reduce reliance on chemical control. Integrated management practices not only improves fruit fly suppression but also contributes to more sustainable and environmentally friendly agricultural systems.

1.2. Problem statement and justification

Fruit flies (Diptera: Tephritidae) are among the most destructive pests of fruits and vegetables worldwide, comprising several genera of major economic importance (Vargas et al., 2015). Among these, *Bactrocera dorsalis* (Hendel), commonly known as the oriental fruit fly, is particularly significant due to its high invasiveness and economic impact. Across Africa, infestations of this species have resulted in substantial socio-economic losses, including export restrictions estimated at approximately US$2 billion annually (Agboka et al., 2024). In Mozambique, *B. dorsalis* is the dominant and most economically important fruit fly species, occurring alongside other genera such as *Ceratitis* (*C. cosyra* and *C. capitata*) and *Dacus* species (*D. bivittatus, D. ciliatus, D. punctatifrons*) (Canhanga et al., 2021).

The wide host range of *B. dorsalis*, includes mango (*Mangifera indica*), guava (*Psidium guajava*), citrus, and various vegetable crops. In the absence of effective management, crop losses can reach up to 80%, depending on agro-ecological conditions and crop type (Agboka et al., 2024). Current management strategies in southern Africa mainly rely on male annihilation techniques (MAT) using parapheromone-baited traps, bait spraying, biological control (using mainly parasitoids), and orchard sanitation (removal of fallen fruits) (Ndlela, 2017). MAT involves deployment of high density trapping stations to reduce male fruit fly populations, while bait sprays (such as GF120 NF and Mozoferm bait) are mainly based on the use of food baits combined with a killing agent and sprayed in the host tree canopy at a weekly basis, to target the females of fruit flies (Ekesi & Billah, 2007). However, the continued prevalence of fruit fly infestations suggests limitations in the effectiveness, adoption, or sustainability of these control methods under local conditions (Kassie et al., 2020).

To address these challenges, alternative and more targeted approaches such as long lasting protein bait-based attract-and-kill systems (e.g., M3 bait stations) have been developed. A bait station is a distinct device that combines bait with a toxicant and is an alternative to bait sprays (Vargas et al., 2015). Although similar protein bait technologies have demonstrated effectiveness in reducing fruit fly populations in various agro-ecological settings and are recommended within integrated pest management programs (Vargas et al., 2015; FAO/IAEA, 2018), their performance under Mozambican conditions remains untested. Therefore, there is a need to evaluate the effectiveness and adaptability of M3 bait stations within local fruit production systems.

In addition, classical biological control efforts against fruit flies in Mozambique began with the introduction of parasitoids *Fopius arisanus* and *Diachasmimorpha longicaudata*, released in Pemba in 2013 (Cugala et al., 2016). Although both species were reported to have established at the release site, with relatively low parasitism rates, there is currently limited information regarding their dispersal and establishment in surrounding areas. Assessing the effectiveness of these parasitoids requires reliable estimates of fruit fly infestation levels across different host fruits and agroecological zones and there is a need to collect comprehensive data on fruit fly infestation levels in the studied areas (Ekesi et al., 2006).

Given these gaps, this study was conducted to evaluate the effectiveness of M3 bait stations, applied alone and M3 in combination with the male annihilation technique and to determine the fruit flies infestation indices. Furthermore, the study aimed to assess the current status and dispersal of *F. arisanus* and *D. longicaudata* from their original release sites to adjacent areas in Cabo Delgado Province.

1.3. Objectives

1.3.1. General objective

To evaluate the effectiveness of fruit fly management strategies involving M3 bait stations and the Male Annihilation Technique, quantify fruit fly infestation levels, parasitism rates and evaluate the establishment and spread of introduced parasitoids.

1.3.2. Specific objectives

- To assess the effectiveness of M3 alone and M3 in combination with male annihilation technique in reducing fruit fly density in Boane District;
- To determine infestation indices of fruit flies and parasitoids parasitism rate on different host fruits in Cabo delgado and Maputo provinces;
- To measure dispersal distance by mapping introduced parasitoids presence relative to the release site in Cabo delgado province.

1.4 Hypothesis

- Orchards treated with M3 bait stations combined with the male annihilation technique exhibit significantly lower *B. dorsalis* populations and fruit infestation levels than orchards treated with M3 bait stations alone or untreated orchards in Boane District;
- There is a significant difference in infestation indices of fruit flies and parasitism rate among different species, hosts and sampled locations;
- The parasitoids *F. arisanus* and *D. longicaudata* have successfully established and dispersed from their initial release sites into adjacent fruit-growing areas of Cabo Delgado Province.

1.5 Study questions

- What is the comparative effectiveness of the three management approaches (M3 + MAT, M3 alone, and untreated control) in suppressing *B. dorsalis* populations in Boane District?
- Do fruit fly infestation indices and parasitism rate vary significantly among different host fruit species and locations?
- What are the dispersal distances achieved by *F. arisanus* and *D. longicaudata* from their initial release locations in Cabo delgado province?

2.0 LITERATURE REVIEW

2.1 Economic importance of fruits production in Mozambique

Fruit production has emerged as a vital sector for economic development in Mozambique, significantly contributing to household incomes and creating employment opportunities, especially in rural areas. The Food and Agriculture Organization (FAO, 2021) emphasizes that the horticultural sector is crucial for food security and nutrition, providing a range of agricultural products that enhance dietary diversity (Abbas & Cabanelas, 2021).

In recent years, Mozambique has observed a growth in horticultural exports, which is driven by favorable agro climatic conditions (World Bank, 2021). The production landscape includes both subsistence farmers and commercial enterprises. Family-owned farms primarily cater to local markets, while larger businesses target export markets, particularly within the Southern African Development Community (SADC), the European Union, and the Middle East Data from the 2022 Agricultural Census indicates that around 75% of rural household incomes stem from crop production, including fruits such as mangoes which are of the most widely cultivated and sold fruits in the country (Baxter et al., 2022). Notably, the mango sector has seen significant growth driven by increasing demand from both domestic and international markets (FAO, 2021).

In the provinces of Cabo Delgado and Manica, it is estimated that approximately 150,000 families are engaged in small-scale mango and banana cultivation. The export potential for these fruits is considerable; however, according to the World Bank's Mozambique Economic Update, the country’s fruit exports to South Africa experienced a substantial decline from nearly USD 60 million in 2014 to about USD 30 million in 2017 (World Bank, 2021). This decrease has largely been attributed to challenges such as the outbreak of Panama disease, which has negatively impacted banana production in northern Mozambique. Since 2018, Mozambique's mango exports have faced restrictions due to the presence of *Bactrocera dorsalis*, a pest that has led to significant export limitations, particularly affecting producers in the Manica province (Cugala et al., 2012). The concentration of large fruit production farms is significant in provinces like Nampula and Manica, where mangoes are increasingly cultivated for export to markets in South Africa and Europe (Canhanga et al., 2021).

2.2 Fruit flies of economic importance

The global diversity of fruit flies has expanded significantly, with over 4300 recognized species and subspecies classified within approximately 500 genera among these, the family

Tephritidae, which belongs to the order Diptera, remains the most economically significant in agriculture (Ndlela et al. 2020). This family is categorized into several subfamilies and tribes, with notable genera, in Africa, including *Ceratitis*, *Dacus*, *Zeugodacus* and *Bactrocera*, with at least six species identified as key pests due to their destructive impact on fruit production. These include *Bactrocera dorsalis* (Oriental fruit fly). *Ceratitis capitata* (Mediterranean fruit fly) *Ceratitis cosyra* (African fruit fly), *Zeugodacus cucurbitae* (Melon fly), and *Dacus ciliatus* (African cucurbit fly) (Ekesi et al., 2016).

In Mozambique, *C. cosyra, C. capitata*, *B. dorsalis* and various Dacus species are significant pests affecting fruits and vegetables yield and quality (Ekesi et al., 2016). *B. dorsalis* poses a substantial quarantine threat due to its rapid spread and severe impact on diverse fruit crops (Niassy et al. 2022). A study conducted in Manica Province, Mozambique, reported that *B. dorsalis* infestations led to average yield losses of 5.65 tons per hectare, equating to a financial loss of approximately USD 3,428.97 per hectare ,the economic injury level was estimated at 33.14 *B. dorsalis* per trap per week, underscoring the species' significant threat to commercial mango production (Canhanga et al., 2020).

Fruit flies are economically important primarily due to their larval development within the fruit, which complicates control measures and leads to substantial economic losses for farmers (Agboka et al., 2022). The larvae feed on the fruit, causing it to rot and making it unmarketable, ultimately affecting both local and export markets (Cugala et al., 2012).

2.3 Origin and distribution of the oriental fruit fly

Bactrocera dorsalis is a phytophagous species native to Asia, and it was first identified on Taiwan Island, China, in 1912 and has since spread throughout the Pacific-Asia region over the past century (Wan et al., 2012). While its origin is confidently traced to Southeast China, the mechanisms driving its expansion into new territories remain partially understood, despite numerous studies (Clarke et al., 2019).

It has become invasive in various regions, including Africa, the Pacific Islands, and parts of the Americas (Figure1). This species has shown remarkable adaptability to diverse climates, which has facilitated its establishment beyond its original habitat. The first confirmed presence in Africa was recorded in Kenya in 2003 (Lux et al., 2003) and since then, it has rapidly proliferated across the continent, In Mozambique, *B. dorsalis* was first detected in 2007 in Cuamba district, Niassa province (Correia et al., 2008).

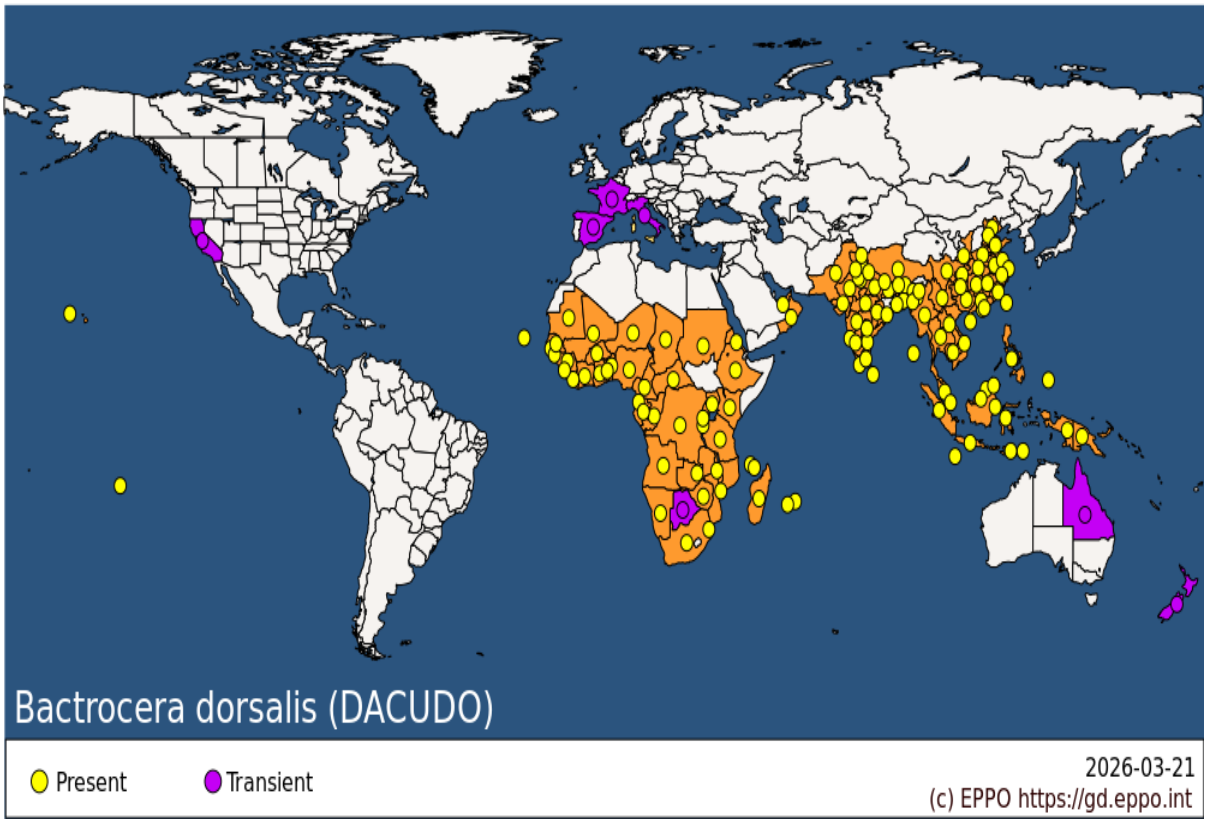


Figure 1. Distribution map of *Bactrocera dorsalis* (EPPO Global Database, 2026).

2.4 Description of *Bactrocera dorsalis*

2.4.1 Biology of *B. dorsalis*

The biology of *B. dorsalis* is characterized by its reproductive behavior and lifecycle adaptations that enhance its survival and proliferation where by female fruit flies have specialized ovipositor that allows them to puncture the skin of fruits, laying an average of 700 eggs directly into the pulp (Weems et al., 2023). The number of eggs can vary depending on the type of host fruit, highlighting the species' adaptability to different environments and food sources (Vargas et al., 2015).

B. dorsalis is classified as a multivoltine species, meaning it can produce multiple generations within a single year (Ekesi et al., 2016). Environmental factors influencing *B. dorsalis* behavior and development play a critical role in its lifecycle (Midingoyi et al., 2021). Temperature, humidity, and the availability of host fruits significantly affect the rate of egg hatching and the survival of larvae (Mohammed et al., 2019). Under optimal conditions, the eggs can hatch within a few days, with larvae immediately burrowing into the

fruit to feed. This feeding behavior not only damages the fruit but also creates entry points for pathogens, further exacerbating crop losses (Vargas et al., 2015).

2.4.2 Life cycle of *B. dorsalis*

Bactrocera dorsalis undergoes complete metamorphosis, consisting of four stages: egg, larva, pupa, and adult (Weems et al., 2012). The entire life cycle spans approximately 40 to 60 days, heavily influenced by climatic conditions and the availability of suitable host fruits the developmental stages include:

Oviposition: Females lay eggs in the fruit, typically at a depth of 2 to 5 mm, depositing 3 to 8 eggs per oviposition event (Figure 2).

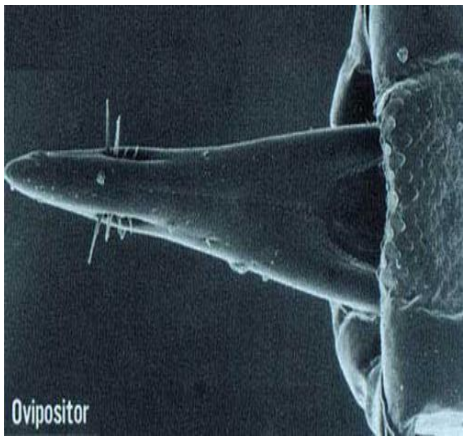


Figure 2. Ovipositor of *Bactrocera dorsalis* (Weems et al., 2012).

The white, elongate and elliptical egg measures about 1.17 x 0.21 mm and has a chorion without sculpturing (Figure 3).

Figure 3. Eggs of *Bactrocera dorsalis* (Weems et al., 2012).

After oviposition, larvae develop within the fruit for 3 to 12 days, the third-instar, which has a typical maggot appearance, is about 10 mm in length and creamy white, the only band of spicules encircling the body is found on the first segment (Clarke et al., 2019). The external part of the anterior respiratory organs, the spiracles, located one on each side of the pointed or head end of the larva, has an exaggerated and deflexed lobe at each side and bears many small tubercles, the caudal segment is very smooth while the posterior spiracles are located in the dorsal third of the segment as viewed from the rear of the larva (Weems et al., 2012). The mature larva emerges from the fruit, drops to the ground, and forms a tan to dark brown puparium about 4.9 mm in length (Figure 4).

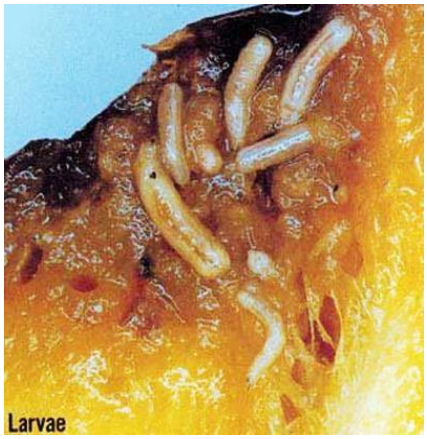


Figure 4. Larvae of the *Bactrocera dorsalis* (Weems et al., 2012).

Once the larvae mature, they exit the fruit and burrow into the soil to pupate, a process that takes approximately 8 to 20 days depending on environmental conditions, during which the pupae vary in color from white to brown and measure about 4–12 mm in length. (Ekesi et al., 2016).

Adult flies emerge from the soil, capable of flight and feeding on sugary substances such as nectar, which is essential for their survival and reproduction becomes sexually active 4 to 10 days later (Weems et al., 2012). Female fruit flies lay eggs just beneath the fruit skin, where larvae (maggots) hatch and feed inside the fruit, causing internal damage (Clarke et al., 2019). Once fully developed, mature larvae leave the fruit, drop to the soil, and form puparia in which pupation occurs, after which adult flies eventually emerge from the pupae (Figure 5) (Ndlela et al. 2020).

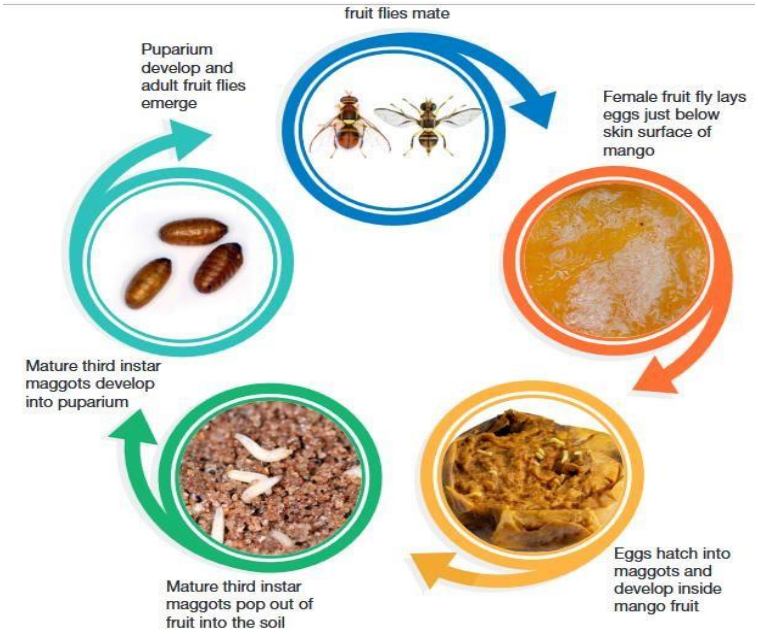


Figure 5. Life cycle of *Bactrocera dorsalis* (Ekesi et al., 2016).

2.4.3 Morphology of *B. dorsalis*

Bactrocera dorsalis adult flies are approximately 8 mm in body length, slightly larger than a housefly, with wings about 7.3 mm long where by the body color is variable but generally bright yellow with a dark "T" shaped marking on the abdomen (Weems et al., 2012). The morphology of *Bactrocera dorsalis* includes distinctive features that make it easily identifiable, with the head characterized by a vertical length of about 1.62 mm, contributing to its unique profile (Vayssières et al., 2012). The dorsal surface of the thorax varies in coloration from red to black and is marked with black stripes that create a strong contrast, while two prominent yellow thoracic lines further enhance its visual distinction (Mohammed et al., 2019).

The oval-shaped abdomen lacks a distinct tergum, which is a notable morphological feature, and instead bears a pecten-like structure that may be associated with reproductive behavior or mating displays (Clarke et al., 2019). These morphological characteristics not only facilitate species identification but also reflect adaptations to its ecological niche as a fruit fly (EPPO, 2021).

The wings of *B. dorsalis* are predominantly transparent, with lengths ranging from 5.4 to 6.9 mm, which allows for agile flight (Figure 6).

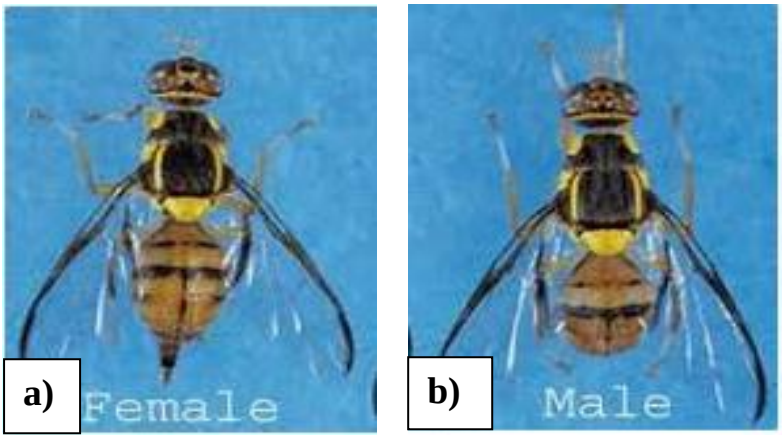


Figure 6. Morphological comparison of adult *Bactrocera dorsalis*: (a) female; (b) male **(**Weems et al., 2012).

2.5 Description of the other fruit flies genera

2.5.1 Genus Dacus

2.5.1.1 Biology of Dacus

Species of *Dacus* (Diptera: Tephritidae) are phytophagous insects whose larvae develop within host plant tissues, particularly fruits, flowers, or stems of both wild and cultivated plants (Vargas et al., 2015). Adult females use their ovipositor to puncture the host tissue and deposit eggs beneath the surface, where the larvae subsequently develop (Ekesi & Billah, 2007). After hatching, larvae feed internally within the fruit, causing tissue breakdown that often leads to premature fruit drop (Ndlela et al. 2020). The life cycle consists of four main stages: egg, three larval instars, pupa, and adult (Kassie et al., 2020). Pupation typically occurs in the soil after mature larvae exit the fruit, and development time varies depending on temperature and host availability (Vargas et al., 2015). Adults feed on nectar, honeydew, and plant exudates for energy and require protein sources for sexual maturation and egg production, also many *Dacus* species are important agricultural pests, especially in tropical and subtropical regions, only 11 species are considered agricultural pests, notably *Dacus puntactifrons*, *Dacus bivittatus*, *D. ciliatus* (Ekesi & Billah, 2007).

2.5.1.2 Morphology of Dacus

Adult *Dacus* flies are medium-sized, often with a slender body and patterned wings typical of tephritid fruit flies (Figure 7). The head has large compound eyes, short antennae with an arista, and well-developed mouthparts adapted for sponging liquids (Vargas et al., 2015).
The thorax is usually marked with distinct color patterns that are often yellow, brown, or black, while the wings commonly display characteristic bands or spots that are useful for species identification, and the abdomen is elongated with females possessing a pointed, sclerotized ovipositor adapted for inserting eggs into plant tissues (Ndlela et al. 2020). Larvae are legless, cylindrical maggots with a tapered anterior end and mouth hooks for feeding, while pupae are enclosed in a hardened, barrel shaped pupation (Ekesi et al., 2020).

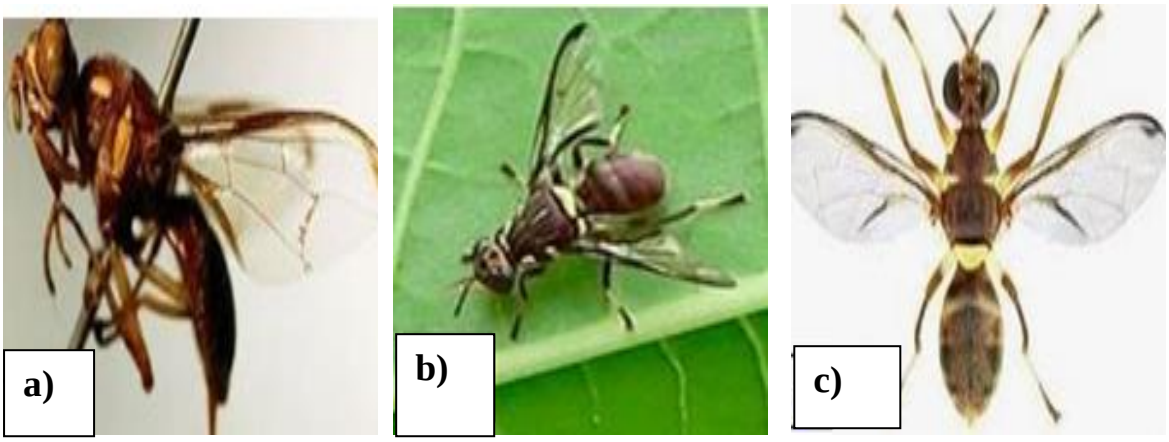


Figure 7. Some economically important Dacus species: (a) *D. ciliatus*; (b) *D. punctatifrons*; (c) *D. vertebratus* (Kassie et al., 2020).

2.5.2 Genus Ceratitis

2.5.2.1 Biology of Ceratitis

Ceratitis species include the economically important *Ceratitis cosyra*, *Ceratitis capitata*, and *Ceratitis rosa*, which are polyphagous fruit flies whose larvae develop within a wide range of host fruits (Midingoyi et al., 2016). Female flies use their serrated ovipositor to puncture fruit surfaces and deposit eggs beneath the skin, where the eggs hatch into larvae that feed on internal tissues, leading to fruit decay, premature fruit drop, and significant yield loss (Mohammed et al., 2019).

The life cycle includes egg, three larval instars, pupa, and adult stages, with soil-based pupation after larvae exit the fruit (Manrakhan & Lux, 2006). Development rates are strongly influenced by temperature and host quality conditions typical in Mozambique's tropical and subtropical climates facilitate multiple generation's annually (Rwomushana et al., 2008). Adults feed on nectar, honeydew, and other plant exudates for energy and require protein-rich diets for reproductive maturation, which explains their abundance in irrigated orchards and mixed cropping systems (Ekesi et al., 2016).

2.5.2.2 Morphology of *Ceratitis*

Adult *Ceratitis* fruit flies are moderate-sized (usually 4–7 mm), with robust bodies and distinctive wing patterns typical of Tephritidae (Figure 8). The head bears large, reddish-brown compound eyes and short three-segmented antennae with an arista (Weems et al., 2012). The thorax often exhibits variable yellow, brown, and black markings, while wings display characteristic dark bands or spots that are useful diagnostic features for species identification. Females possess a well-sclerotized, telescoping ovipositor adapted for fruit penetration during oviposition and the abdomen is segmented and tapered, sometimes with color pattern variation among species and sexes (Rwomushana et al., 2008). Immature stages legless larvae are creamy white maggots with tapered anterior ends and mouth hooks used for internal feeding; pupae are brown, barrel-shaped puparia where metamorphosis occurs in the soil (EPPO, 2021).

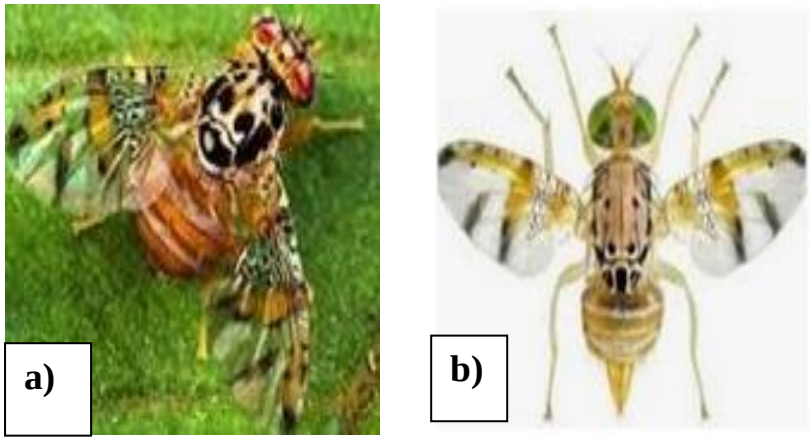


Figure 8. Some economically important Ceratitis species: (a) *C. capitata*; (b) *C. cosyra* (Vargas et al., 2015).

2.6 Fruit flies host plants and damage

2.6.1 Host plants

Fruit flies have been reported to infest hundreds of host plant species across numerous plant families, with some species attacking over 400 host plants worldwide (EPPO, 2026). The important affected crops include:

- Anacardiaceae: mango (*Mangifera indica*)
- Rutaceae: citrus (*Citrus reticulata*)
- Myrtaceae: guava (*Psidium guajava*)
- Lauraceae: avocado (*Persea americana*)
- Musaceae: banana (*Musa acuminata*) Solanaceae: tomato (*Solanum lycopersicum*)
- Cucurbitaceae: pumpkin (*Cucurbita pepo*) melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*)
- Bromeliaceae: pineapple (*Ananas comosus*).

These extensive hosts range are not only facilitate the establishment of stable populations but also enable the species to thrive in varying environmental conditions throughout the year; consequently, this adaptability complicates control efforts aimed at mitigating its impacts on agricultural production (Vargas et al., 2015).

2.6.2 Damage caused by fruit flies

Damaged caused by fruit flies can be categorized into direct and indirect effects (Cugala et al., 2016). Direct damage occurs when the female fruit flies pierces the skin of the fruit to lay their eggs, resulting in scars that diminish the fruit's quality, once the larvae hatch, they burrow into the fruit pulp, creating holes and tunnels that accelerate its decay and spoilage (Figure 9) (Cugala et al., 2016).

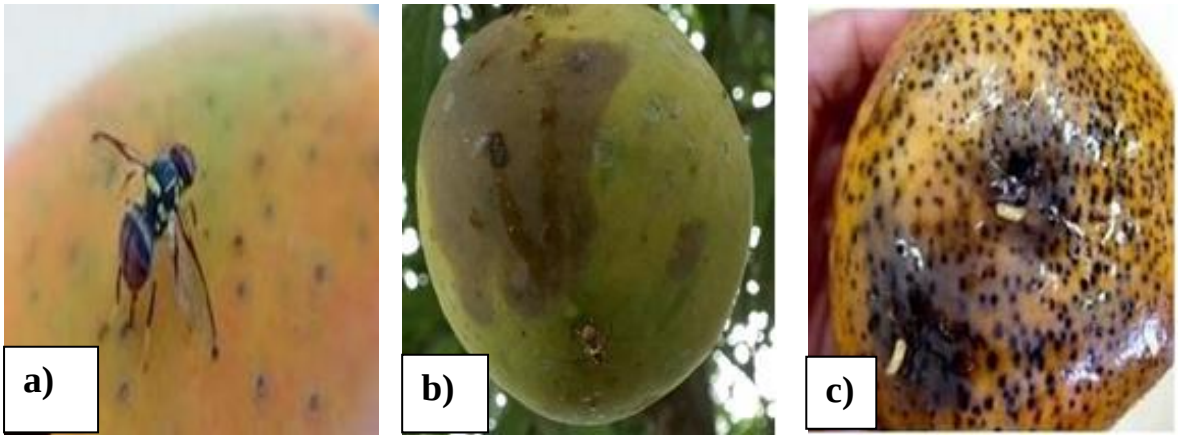


Figure 9. Direct damage caused by fruit flies: (a) female fruit fly ovipositing in mango; (b) rotting tissues in damaged mango surface; (c) larvae of fruit flies on fruit (Kassie et al., 2020).

Fruit flies cause significant damage to mango production in Africa. In regions where *B. dorsalis* is present, particularly high losses have been reported. For instance, the percentage of damaged mango fruits in Mozambique ranged from 21% to 78% due to country's diverse agro-ecological conditions, particularly the more humid northern regions, create favorable environments for higher fruit fly populations (Canhanga et al., 2020). In addition, mango production is largely managed by smallholder farmers, whose orchard management practices and access to pest control methods vary considerably and most farmers not follow proper sanitation like clean fallen fruits, prune trees, and use bait or other insect control methods. The continuous availability of host fruits such as mango, cashew, and citrus also supports year-round breeding of fruit flies. Furthermore, the widespread presence of *Bactrocera dorsalis*, a highly invasive and dominant species, significantly increases infestation levels. The uneven distribution and limited effectiveness of natural enemies, such as parasitoids, in some areas also influence the extent of damage (Canhanga et al., 2020). Similar studies in Mozambique have reported high levels of mango fruit damage due to *Bactrocera dorsalis*, ranging from about 41% to over 77% in Manica Province, mainly attributed to favorable climatic conditions (warm temperatures and high humidity), which enhance fruit fly survival and reproduction, as well as the continuous availability of host fruits such as mango and other alternative hosts in the surrounding environment (Cugala et al., 2016). In addition, limited and inconsistent application of integrated pest management practices among smallholder farmers further contributes to high infestation levels. While some reports from northern regions indicate losses reaching up to 94% in unprotected orchards (Cugala et al., 2016), this extreme damage is largely associated with the absence of control measures, poor orchard

sanitation, and high population pressure of *B. dorsalis* in highly suitable agro-ecological zones. In addition to direct damage, indirect losses are also considerable and are mainly associated with commercialization restrictions. Because *B. dorsalis* is classified as a quarantine species, importing countries impose strict phytosanitary measures to prevent its introduction and establishment, leading to significant export constraints. Data released by the CTA (2020) indicated that mango losses due to infestation by this species can reach up to 80% by the end of the harvesting season. During a one-month suspension of mango exports to the South African market, losses were estimated at US$2.5 million (Cugala et al., 2011).

According to Kassie et al. (2020), the damage caused by fruit flies can be classified as economic, social, and environmental impactsEconomic losses are associated with increased production costs due to the use of control measures, pest monitoring, and costs related to post-harvest treatments, which make orchards unprofitable and may lead to loss of investment and producer failure (Agboka et al., 2022). For instance, a study on the economic impact of apple maggot infestation in Washington State estimated that the spread of this pest could result in losses ranging from $510 million to $557 million, depending on pest pressure (Ekesi et al., 2016).

The inability to sell fruits in international markets limits generation of foreign exchange for the country, and fruit from infested areas often commands lower prices at domestic market resulting loss of markets and reduced tax revenue (Cugala et al., 2011).

Social losses associated with fruit flies can significantly decrease crop yields and quality, leading to diminished income for farmers, therefore this decline may result to increased unemployment and economic instability in agricultural regions (Midingoyi et al. 2016). As income falls, families may abandon fruit farming, contributing to rural exodus as individuals seek better opportunities in urban areas (Rwomushana et al., 2008). Additionally, the decline in fruit production can disrupt local food security, affecting community access to fresh produce and increasing reliance on imported goods (Kassie et al., 2020).

Finally, environmental losses are associated with the use of chemical pest control that can contaminate the environment and other non-target organisms, the application of pesticides can lead to contamination of soil, water sources, and air, posing significant risks to the surrounding ecosystem (Bota et al., 2018). These chemicals may adversely affect beneficial insects, birds, and aquatic life, thereby disrupting local biodiversity and altering food webs (Ndlela et al., 2020). Additionally, pesticide runoff can contaminate nearby water bodies,

impacting aquatic ecosystems and potentially entering the human food chain (Mwatawala et al., 2006). The long-term reliance of chemical controls can also lead to the development of pesticide-resistant fruit fly populations, prompting farmers to increase application rates or seek even more harmful alternatives (Kassie et al., 2020).

2.7 Monitoring of fruit flies

Monitoring and surveillance of fruit flies using attractant trap**s** is the systematic use of baited traps to detect, quantify, and track fruit fly populations in orchards or other host areas. These traps use chemical lures (male-specific or food-based attractants) to capture flies, allowing farmers to assess population density, detect early infestations and evaluate the effectiveness of management strategies (Vargas et al., 2015).

2.7.1 Chemical male specific lures

These are species specific attractants, male-oriented such as methyl eugenol or cue-lure, which chemicals target male sensory receptors, drawing them into the trap.

- Methyl eugenol (4-allyl-1, 2-dimethoxy-benzene) – attracts males of most Bactrocera species**.** It is formulated as polymeric plugs for slow release rates that can stay active in the field for 4-6 weeks. An insecticide must be used to kill the attratect flies. It can be used with in McPhail or tephri traps (Kassie et al., 2020).
- Cuelure (4-(p-acetoxyphenyl)-2-butanone) - Males of other important Bactrocera, Zeugodacus and some Dacus species are attracted to this lure. It is formulated as polymeric plugs for slow release that can stay active in the field for 4-6 weeks. An insecticide must be used to kill the attracted flies. It can be used with in McPhail or tephri traps (Kassie et al., 2020).

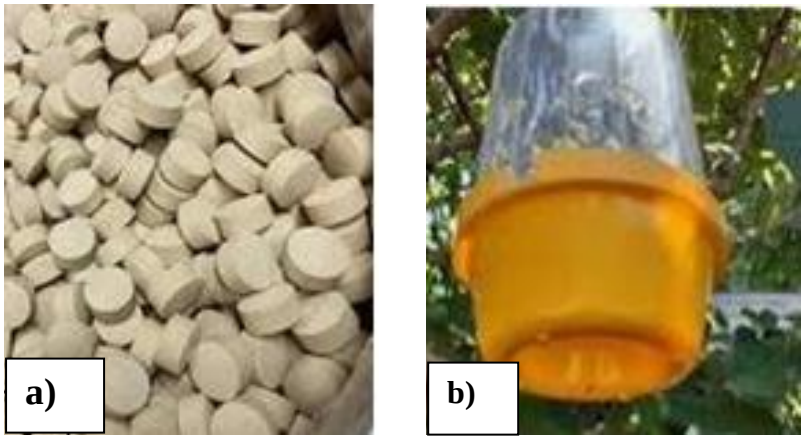
2.7.2 Food based attractants

2.7.2.1 Torula yeast

Torula yeast is a protein-based attractant widely used in fruit fly management due to its strong appeal to female fruit flies, although it can also attract males to a lesser extent. It is particularly effective for monitoring population density and detecting early infestations in orchards, as adult flies are naturally drawn to protein sources required for reproductive development. Torula yeast is commonly formulated as tablets that are dissolved in water and

deployed in traps such as McPhail or Tephri traps (FAO/IAEA, 2018). Under normal field conditions, the solution is typically replaced every 7 to 14 days to maintain its effectiveness. In addition, it is known to attract a wide range of fruit fly species, as well as some non-target insects such as other Diptera and Lepidoptera (Ekesi et al., 2016).

Several studies have further demonstrated the effectiveness of Torula yeast in fruit fly management across different regions. For instance, in Kenya, Ekesi et al. (2014) reported that Torula yeast traps captured significantly higher numbers of fruit flies, particularly females, compared to other protein-based attractants such as hydrolyzed protein baits. Similarly, in South Africa, Manrakhan et al. (2017) found that Torula yeast consistently recorded high capture rates across different agro-ecological conditions, outperforming several commercially available food-based attractants and demonstrating its reliability under varying environmental settings. In Hawaii (USA), Vargas et al. (2001) observed that Torula yeast-based trapping systems, when applied continuously in orchard conditions, contributed to a clear reduction in fruit fly populations over time compared to untreated orchards. The effectiveness of Torula yeast in these studies is mainly attributed to its release of ammonia and other volatile compounds during fermentation, which mimic natural food sources and strongly attract protein-seeking female flies (FAO/IAEA, 2018). Also, its stability under field conditions and its ability to remain active for extended periods enhance its performance, making it a dependable tool for monitoring and reducing fruit fly populations and associated fruit damage (Figure 10)(Vayssières et al., 2012).



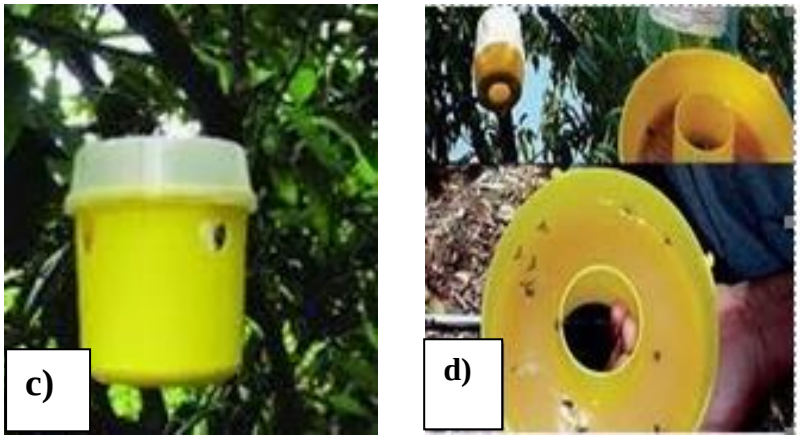


Figure 10. Atractant and different traps used for fruit flies monitoring: (a) torula; ((b) Mcphail trap; (c) Tephri trap and (d) Torula effectively attracting fruit flies (Vargas et al., 2015).

2.7.2.2 Biolure

Biloure contain synthetic formulations designed to mimic the volatiles emitted by ripe or fermenting fruits, which attract fruit flies searching for feeding and oviposition sites, being appealing to females fruit flies but can also attract males (less effective) (Ndlela et al., 2020). It is commercially produced in solid formulation that are stable and long lasting in field conditions, with recommend replacing at intervals of 4 up 6 weeks under tropical conditions (Vargas et al., 2015). An insecticide must also be placed in the trap to kill the attracted flies or a sticky trap must be used. It targets *B.dorsalis*, *C. capitata*, and other tephritid pests. It can be used with in McPhail or tephri traps (Figure 11).

Many studies have demonstrated the effectiveness of Biolure in fruit fly management across different regions, including Africa, particularly due to its strong attraction to female populations. For example, in Spain, Navarro-Llopis et al. (2013) reported that Biolure traps captured significantly higher numbers of *Ceratitis capitata* compared to ammonium acetate-based attractants, with improved selectivity for females. Similarly, in Israel, Heath et al. (2004) found that Biolure, composed of ammonium acetate, trimethylamine, and putrescine, resulted in higher capture rates of female fruit flies compared to standard food-based lures, leading to more effective monitoring and population suppression. In Mexico, Robacker (2001) also showed that Biolure-based trapping systems significantly reduced fruit fly populations over time when compared to untreated controls and some conventional attractants.

In the African context, Ekesi et al. (2014) in Kenya reported that Biolure traps were highly effective in capturing *Bactrocera dorsalis* and other tephritid species, performing better than some locally available food-based attractants in terms of female captures. Likewise, Manrakhan et al. (2017) in South Africa found that Biolure maintained consistent and relatively high capture rates under different environmental conditions, demonstrating its suitability for use in diverse agro-ecological zones and outperforming several alternative attractants in long-term monitoring programs.

The superior performance of Biolure in these studies is mainly attributed to its synthetic composition, which releases a combination of volatile compounds such as ammonia and amines (ammonium acetate, trimethylamine, and putrescine) that closely mimic odors from ripe and fermenting fruits, thereby strongly attracting protein-seeking females (Vayssières et al., 2012). Furthermore, its controlled and long-lasting release mechanism ensures stable performance under field conditions, allowing it to outperform other attractants that degrade more quickly or produce less consistent signals (Muhammad et al., 2023).

Based on these findings, the consistent effectiveness of Biolure across different studies highlights its reliability as a standard attractant for fruit fly monitoring and management programs. Its ability to selectively target female fruit flies particularly *Bactrocera dorsalis* makes it especially valuable, as it directly relates to population suppression by focusing on the reproductive segment of the population. Studies such as Ekesi et al. (2014), Manrakhan et al. (2017), and Vargas et al. (2015) confirm that Biolure not only enhances trap sensitivity but also provides more accurate population estimates compared to conventional protein-based lures. This overall performance demonstrates that Biolure is a robust and scientifically validated tool suitable for integration into integrated pest management (IPM) strategies, particularly in tropical and subtropical regions where fruit fly pressure is high. Moreover, its standardized formulation ensures reproducibility of results across different studies and locations, which is essential for comparative research and large-scale pest surveillance. In addition, Biolure's reduced variability in capture rates over time improves the reliability of long-term monitoring data. Its compatibility with different trap types further enhances its practicality for field application (Muhammad et al., 2023).The reduced need for frequent replacement compared to some food-based attractants also contributes to lower labor and maintenance costs. Altogether, these advantages reinforce Biolure's position as an effective and dependable attractant for sustainable fruit fly management programs (Vayssières et al., 2012).

Figure 11. Consumables used for fruit flies monitoring: (a) Packaging of Biolure; (b) dichlorvos (DDVP); (c) Mcphail trap containing Biolure and dichlorvos (DDVP); (d) Biolure effectively attracting fruit flies (FAO/IAEA, 2018).

2.8 Fruit fly control methods

The purpose of the control methods is to reduce yield losses in fruits and vegetables due to fruit fly infestation, while also promoting both the internal and external fruit quality of fruits (Ndlela et al., 2020). Numerous strategies have been documented for controlling fruit flies,, including bait application (Cugala et al., 2016), traps and attractant management, sanitation practices (FAO, 2024), mechanical protection of fruits using newspaper or paper bags, timely harvesting. Postharvest treatments (FAO, 2024), sterile insect technique and biological control (FAO/IAEA, 2018).

Chemical control remains an important component of fruit fly management, especially when rapid population suppression is required to protect commercial fruit production (Hassani et al., 2022). Modern chemical control strategies aim to reduce fruit fly populations while minimizing environmental impact and reducing broad insecticide spraying (Moquet et al.,

2024). That is why targeted bait and lure techniques such as the Male Annihilation Technique (MAT) and protein bait stations are widely used.

2.8.1 Male Annihilation Technique (MAT)

It is a widely used chemical control method for fruit flies, which combines male attractants with insecticide to reduce male populations (Vargas et al., 2013) The male annihilation technique (MAT) is highly species-specific, as it relies on male lures such as methyl eugenol, which primarily attracts species like *Bactrocera dorsalis* (Ekesi et al., 2016). When MAT is used alone, it may not effectively control all fruit fly species present, and it does not directly target females. The technique is particularly effective for species whose males are strongly attracted to lures such as methyl eugenol or cue-lure, allowing targeted suppression of the reproductive population (FAO/IAEA, 2018). The lures mimic natural attractants such as floral or pheromone signals, ensuring that males are drawn away from females and increasing the effectiveness of population suppression (Figure 12) (Vargas et al., 2013).

In practice, MAT is applied widely in orchards using traps or bait stations that contain the lure insecticide mixture and its success depends on area wide coverage, correct placement of traps, and deployment during peak male activity periods (FAO/IAEA, 2018). MAT devices remain effective for several weeks (6-8 weeks) and therefore requires periodic replacement because the attractant gradually evaporates and the toxicant loses effectiveness due to exposure to sunlight, rainfall, and high temperatures (Ndlela et al., 2016).

Previous studies have shown that the Male Annihilation Technique (MAT) is highly effective in suppressing fruit fly populations, particularly *Bactrocera dorsalis*, across different regions, including both international and African contexts. For example, in Hawaii (USA), Vargas et al. (2010) reported that the deployment of methyl eugenol combined with a toxicant resulted in a reduction of male populations by over 90%, leading to a significant decline in overall fruit fly density compared to untreated orchards. In Thailand, Tan and Nishida (2012) found that repeated application of MAT blocks achieved rapid suppression of male flies and reduced infestation levels when compared to conventional bait spray programs. Similarly, in Australia, Stringer et al. (2017) demonstrated that MAT grids provided more effective area-wide population control than trapping alone, with sustained reductions observed over several weeks.

In Africa, comparable results have been reported. In South Africa, Ndlela et al. (2016) showed that orchards treated with MAT experienced substantially lower infestation levels, with fruit damage reduced by up to 18–25 times compared to untreated control orchards. In Tanzania, Mwatawala et al. (2006) observed that methyl eugenol-based traps captured significantly higher numbers of male *B. dorsalis* than food-based attractants, confirming its superior targeting efficiency. Furthermore, Manrakhan et al. (2011) reported that area-wide application of MAT in South Africa led to strong population suppression and, in some cases, local eradication when compared to conventional monitoring or trapping approaches alone.

The strong performance of MAT across these studies is primarily attributed to the high specificity and powerful attraction of methyl eugenol to male flies, enabling their rapid removal before mating occurs and thereby disrupting reproduction (Kassie et al., 2020).In addition, the incorporation of a toxicant ensures immediate mortality upon contact, while strategic deployment in grids or repeated applications enhances coverage and maintains continuous pressure on the population. Compared to other control approaches evaluated in these studies, MAT consistently achieved faster and more pronounced population suppression due to its targeted action on the reproductive component of the pest population (Muhammad et al., 2023).

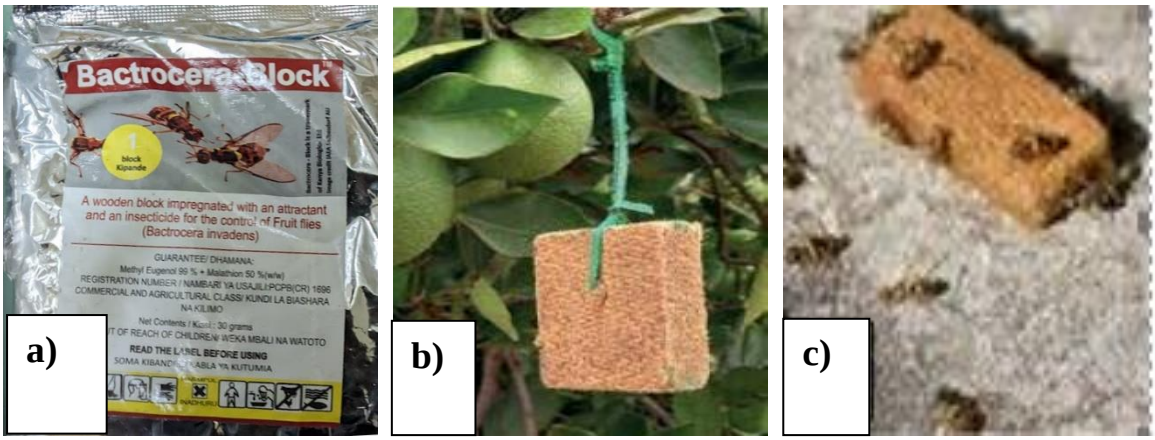


Figure 12. MAT blocks: (a) Packaging of the Bactrocera Block; (b) Bactrocera Block suspended in a mango tree, (d) Bactrocera Block effectively attracting fruit flies (Muhammad et al., 2023).

2.8.2 Protein bait suppression using M3 bait stations

Protein bait based suppression methods are widely used in fruit fly management as a targeted alternative to broad insecticide spraying (Vargas et al., 2015). These methods exploit the natural need of adult fruit flies, particularly females, to obtain protein necessary for egg development. Protein bait formulations mixed with small quantities of insecticide attract flies to feeding points where they ingest the toxicant and die. Because both males and females are attracted, protein bait techniques directly reduce the reproductive population and help lower fruit infestation levels (Muhammad et al., 2023).

M3 bait stations are a form of protein bait delivery system designed to provide a continuous food attractant combined with insecticide in orchards. Adult flies are attracted to the bait source, feed on it, and are subsequently killed, reducing local population density. Compared to foliar insecticide sprays, bait stations concentrate insecticide use at specific points, reducing environmental contamination and preserving beneficial insects while still achieving population suppression (Vargas et al., 2015) (Figure 13).

Previous studies have demonstrated the effectiveness of protein bait stations using M3 formulation in reducing fruit fly populations across different agro-ecological zones, particularly in Africa. For instance, in Burkina Faso, Zida et al. (2023) reported that orchards treated with M3 bait stations showed a significant reduction in *Bactrocera dorsalis* and *Ceratitis cosyra* populations, with infestation levels decreasing by more than 60–80% compared to untreated control orchards. Similarly, Ekesi et al. (2016) working across West and East Africa observed that the use of protein bait stations contributed to maintaining fruit fly populations below economic threshold levels, with fruit damage reduced to approximately 6.7–20% in treated plots compared to over 60% in untreated fields. In Kenya, field evaluations also showed that M3 bait deployment resulted in consistently lower fruit infestation and reduced fly density compared to farmer practice orchards without bait stations (Kassie et al., 2020).

The superior performance of M3 bait stations in these studies is mainly attributed to the protein hydrolysate formulation, which releases ammonia-based volatiles that strongly attract adult fruit flies, particularly protein-seeking females (Kassie et al., 2020). Once attracted, flies are exposed to an insecticidal component (alpha-cypermethrin), leading to rapid mortality through an attract-and-kill mechanism. In addition, the bait stations provide a

protected and long-lasting delivery system, which maintains attractiveness under field conditions and reduces wash-off or rapid degradation (Vargas et al. 2013). Compared to untreated orchards and conventional farmer practices used in these studies, M3 bait stations consistently achieved higher suppression levels due to their combination of strong attraction, targeted killing action, and sustained field stability (Ndlela et al., 2021).

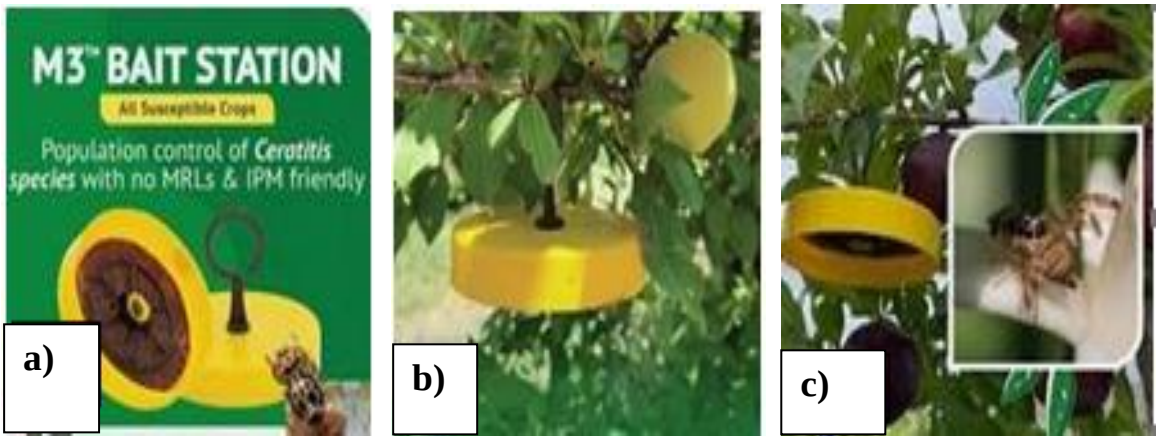


Figure 13. M3 bait station: (a) labeling of M3; (b) M3 is suspended from a Guava branch, effectively positioned for pest attraction; (c) Ceratitis species attracted to the device (Muhammad et al., 2023).

Despite their advantages, bait stations require proper maintenance and replacement of bait materials to remain effective and population reduction also depends on adequate station density and orchard coverage (Niassy et al. 2022).In hot or rainy areas, bait may dry or degrade faster, requiring replacement every 4 up to 6 weeks, whereas in cooler or dry conditions bait can remain effective (Muhammad et al., 2023).

2.8.3 Biological control

Biological control is defined as the use of living natural enemies such as predators, parasitoids, and pathogens to control pest populations, thereby reducing their density to levels that do not cause economic harm (Mohammed et al., 2019). From the point of view of fruit flies egg parasitoids, *Fopius arisanus* are considered more effective than larval parasitoids like *Diachasmimorpha longicaudata* against fruit flies such as *B. dorsalis* (Vayssières et al., 2012).

In Mozambique, the first mass release of the introduced parasitoid *F. arisanus* took place on August 23, 2010, in Niuji (Mieze) and Metuge located in Pemba district of Cabo Delgado Province, northern Mozambique, during this event, 6,000 adult *F. arisanus* parasitoids were

released as a biological control agent to naturally reduce the population of fruit fly pests (Cugala et al., 2016). On September 25th of the same year, the second release of the parasitoid was carried out in the same place with around 2000 adult *F. arisanus* released and in November 2010 the third version of the same parasitoid. Overall, a total of about 18,000 adult *F. arisanus* they were released in Mieze (Cumbe & Cugala, 2014).

In the situation of tephritid fruit flies in Mozambique and other parts of sub-Saharan Africa, native parasitoids such as *Psyttalia cosyrae*, *Utetes africanus* (Figure 14) and other braconid wasps have been recorded attacking larvae of indigenous fruit fly species (Vayssières et al., 2012).

Figure 14. Morphological characteristics of Psyttalia species (Vayssières et al., 2012).

The parasitism rates and effectiveness of parasitoids are shaped by ecological factors including host density, habitat structure, availability of alternative hosts and agricultural practices (Agboka et al., 2022). For example, studies in Kenyan and Tanzanian orchard systems reported parasitism rates by native braconid parasitoids ranging from low to moderate levels, often influenced by fruit fly host abundance and pesticide use (Muhammad et al., 2023).

Diversified agroecosystems that provide floral resources and refuges for adult parasitoids tend to support higher parasitism rates, while orchards with frequent broad-spectrum insecticide applications show reduced parasitoid populations (Vayssières et al., 2012). Among the predators, the African weaver ant (*Oecophylla longinoda*) is utilized as a biological control agent to protect mango and citrus fruits from fruit fly infestations (Agboka et al., 2022).

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2.8.3.1 Description of the parasitoid *Fopius arisanus*

It is an endoparasitoid (an egg–larval parasitoid) originating from the Indo-Australian region, recognized for its effectiveness in controlling fruit fly populations. The species was first successfully introduced and established in Hawaii in 1947 as part of early biological control programs against fruit flies, where it became one of the most effective parasitoids of *Bactrocera dorsalis* (Clausen, 1978). Following its success in Hawaii, *F. arisanus* was later introduced into Africa through coordinated area-wide fruit fly management programs led by international research institutions, particularly the International Centre of Insect Physiology and Ecology (ICIPE, 2023). Large-scale releases in Africa began in Kenya in 2006–2007, where successful establishment and field recovery were first confirmed in mango agro-ecosystems (Mohamed et al., 2010; Ekesi et al., 2016). After establishment in Kenya, the parasitoid subsequently dispersed and was also reported in other African countries including Tanzania starting from around 2008, Uganda, Benin, Cameroon, and Togo, where field parasitism of fruit fly larvae in mango orchards was consistently recorded (Gnanvossou et al., 2016; Ekesi et al., 2016).

In Mozambique, *F. arisanus* was introduced later as part of regional biological control expansion programs supported by ICIPE and national agricultural research systems. The first releases in Mozambique were conducted around 2011–2012, initially in the southern region, particularly Maputo Province, where mango production is concentrated and fruit fly pressure is high (Ekesi et al., 2016). Subsequent monitoring confirmed establishment and dispersal into central zones, indicating adaptation to local agro-ecological conditions, and its establishment in Mozambique was facilitated by the continuous availability of host species such as *Bactrocera dorsalis*, favorable climatic conditions, and suitable mango production systems that support year-round host parasitoid interaction (Mohamed et al., 2010).

This parasitoid specifically targets the eggs and young first-instar larvae of the host, *B. dorsalis* where by female *F. arisanus* parasitoids use their ovipositors to check inside carefully host-infested fruits, laying eggs directly into the eggs of fruit flies (Midingoyi et al., 2016). The parasitoid larva develops within the host, consuming its tissues as it grows through three instars. In the first instar, the larva hatches and begins feeding within the host egg (Kassie et al., 2020). As the host progresses to the larval stage, the larva continues to develop in the second instar. Finally, the third instar occurs within the host pupa, where the parasitoid larva occupies the entire interior, ultimately leading to the death of the host

(Goerge et al., 2011). This developmental process effectively suppresses fruit fly populations by preventing the emergence of adult pests where by the adult emerges from the pupa 18-20 days after oviposition, this happens approximately 2 days after the emergence of adult flies that have not been parasitized (Vargas et al., 2015).

Adult *F. arisanus,* a parasitoid wasp measures approximately 4 millimeters in length, making it relatively small compared to other parasitoids, the body of this species is characterized by a striated appearance, which enhances its visual identification (Figure 15) (Vargas et al., 2013). The coloration of *F. arisanus* contributes to its recognition in the field. The head and thorax are typically dark, providing a stark contrast to the paler coloration of the legs (Kassie et al., 2020). One of the most distinctive features of *F. arisanus* is its narrow ovipositor tip, which is specialized for laying eggs inside the host larvae, this adaptation is critical for the wasp's reproductive success, allowing it to effectively deposit its eggs in the appropriate location for optimal larval development (Mohammed et al., 2019).

Several studies have demonstrated the successful establishment and performance of *Fopius arisanus* in several African countries, particularly as a key biological control agent of *Bactrocera dorsalis*. In Kenya, Ekesi et al. (2016) reported that following field releases, the parasitoid became well established in mango agro-ecosystems, with parasitism rates ranging from approximately 20% to over 50% depending on locality and season, indicating strong adaptation and persistence. Similarly, in Benin, Gnanvossou et al. (2016) observed successful establishment of *F. arisanus* in mango orchards, with parasitism levels reaching about 30–45% under field conditions, and evidence of natural dispersal beyond initial release sites. In Tanzania, Mohamed et al. (2010) further confirmed its establishment and spread across mango-growing regions, where consistent recovery of parasitoids from infested fruits demonstrated its ability to colonize new environments and maintain populations over time (Kassie et al., 2020).

The higher performance of *F. arisanus* compared to other parasitoids is mainly attributed to its unique biological strategy as an egg–larval endoparasitoid, allowing it to attack fruit fly hosts at an earlier developmental stage before larval damage occurs inside the fruit (Vargas et al., 2013). In addition, its strong host-searching ability, high reproductive potential, and capacity to adapt to diverse agro-ecological conditions enhance its establishment success and dispersal ability, and compared to larval parasitoids evaluated in similar studies, *F. arisanus*

consistently shows higher establishment stability and broader spatial distribution, making it more effective in long-term suppression of fruit fly populations (Ndlela et al., 2021)

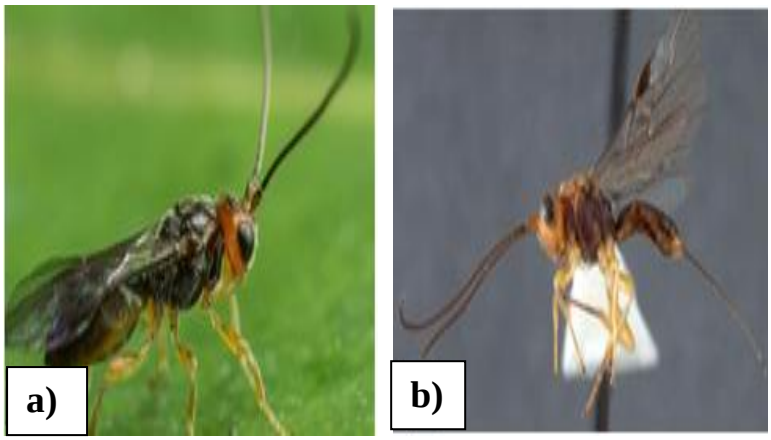

Figure 15. Morphology of *Fopius arisanus*: (a) adult Male; (b) Adult Female (Vayssières *et al*., 2012).

2.8.3.2 Description of the parasitoid *Diachasmimorpha longicaudata*

Diachasmimorpha longicaudata (Hymenoptera: Braconidae) is native to Southeast Asia and subtropical regions, characterized as a solitary species of parasitoid wasp and has been introduced globally as a biocontrol agent against tephritid fruit flies, particularly those in the genus Bactrocera (Clarke et al., 2010). It also parasitizes indigenous *Ceratitis cosyra* (Kassie et al., 2020). In Africa, the parasitoid was first imported and released as part of classical biological control programs targeting invasive fruit flies such as *Bactrocera dorsalis*, with initial introductions reported in East Africa, particularly Kenya, around 2008 following laboratory evaluations and regulatory approval for field release (Mohamed et al., 2008; Ekesi & Hanna, 2008) . These early releases in Kenya demonstrated successful establishment potential and encouraged further dissemination to other African regions, including West and Southern Africa, under coordinated biological control programs (Mohamed et al., 2008).

In Mozambique, *Diachasmimorpha longicaudata* was introduced and field-released as part of regional fruit fly management initiatives implemented under wider African classical biological control programmes targeting *Bactrocera dorsalis*. Documented releases occurred around 2013, particularly in major mango production areas of the northern and central regions, including Cabo delgado, Nampula, Zambézia, and Manica provinces. Following release, the parasitoid was first reported to establish in northern and coastal production zones, especially in areas with high fruit fly pressure and favorable climatic conditions such

as Nampula and Zambézia, where optimal temperature and continuous host availability support sustained population development (Ekesi et al., 2008; Mohamed et al., 2008). Climatic suitability assessments and field evaluations further confirmed that Mozambique provides highly suitable agro-ecological conditions for the establishment and persistence of the species, enabling the development of permanent populations after release (Cugala et al., 2013; Ndlela et al., 2021).

Its effectiveness stems from its ability to parasitize late larval and pupal stages of fruit flies, where it lays its eggs inside third-instar (late-stage) larvae of the host fruit flies, then the parasitoid's eggs hatch within the host, and the developing parasitoid larvae consume the host tissues, ultimately leading to the host's death (Paladino et al., 2010). Under controlled environmental conditions of 25°C, 85% relative humidity, and an 18:6 light-dark photoperiod, the preimaginal development of *D. longicaudata* takes about 16 days where by relatively quick lifecycle allows *D. longicaudata* to produce multiple generations within a growing season, enhancing its potential as a biological control agent (Paladino et al., 2010).

Diachasmimorpha longicaudata adults are relatively small, measuring approximately 4 to 5 millimeters in length (Vargas et al. 2013). Its coloration of is typically dark, complemented by yellow or pale markings on the legs and antennae, which color variation provides some degree of camouflage within their natural environments, helping them blend in among foliage and fruit (Kassie et al., 2020).One of the most notable features of *D. longicaudata* adults is their long and slender ovipositor, which is specifically adapted for laying eggs deep within the host fruit and its specialized structure enables the wasp to insert its eggs into the larvae or pupae of fruit flies, maximizing the chances of successful parasitism (Figure 16) (Vayssières et al., 2012).

Several studies conducted in Africa and other tropical regions have demonstrated the strong biological control potential of *Diachasmimorpha longicaudata* against invasive fruit flies, particularly *Bactrocera dorsalis* (Agboka et al., 2024). In Kenya, the parasitoid was first introduced from Hawaii under classical biological control programs led by ICIPE and released in mango-growing regions, where it successfully established under field conditions (Mohamed et al., 2008; Ekesi et al., 2008). Subsequent field evaluations showed establishment rates associated with parasitism levels reaching up to 17% for *D. longicaudata* under open field conditions, while higher parasitism was observed in more favorable ecological zones when host availability was high (Ndlela et al., 2017). Dispersal studies in Kenya further revealed that the parasitoid can spread naturally beyond release sites, with

recovery recorded up to 20–30 km from the original release point within two years, indicating strong dispersal ability and adaptation to heterogeneous agro-ecosystems (Ndlela et al., 2017). In East and Southern Africa, including Mozambique, climatic suitability models have confirmed that countries such as Kenya, Tanzania, Malawi, and Mozambique provide highly favorable conditions for establishment, supporting permanent populations due to continuous host availability and optimal temperatures (Ekesi et al., 2011; Ndlela et al., 2021). In Mozambique specifically, field releases conducted around 2013 in major mango production zones (Nampula, Zambézia, and Manica) resulted in successful establishment under tropical conditions similar to those observed in Kenya, reinforcing the parasitoid's adaptability across Africa (Cugala et al., 2013).

The high performance of *D. longicaudata* compared to many indigenous parasitoids is mainly attributed to several biological and ecological advantages (Agboka et al., 2024). This is because it is a highly efficient koinobiont larval prepupal endoparasitoid, allowing it to attack fruit fly larvae inside the fruit and continue development as the host grows, which increases survival success (Ndlela et al. 2020). It exhibits strong host-searching and foraging ability, with studies showing higher host probing and searching rates compared to competing parasitoids such as *Psyttalia cosyrae* (Ekesi et al., 2020). Also, it has a relatively wide host range among tephritid fruit flies, enabling persistence even when a single host species fluctuates, its high dispersal capacity and climatic adaptability allow it to colonize new areas rapidly after release, contributing to long-term establishment and suppression of fruit fly populations across Africa (Mohamed et al., 2008; Ekesi et al., 2011; Ndlela et al., 2017).

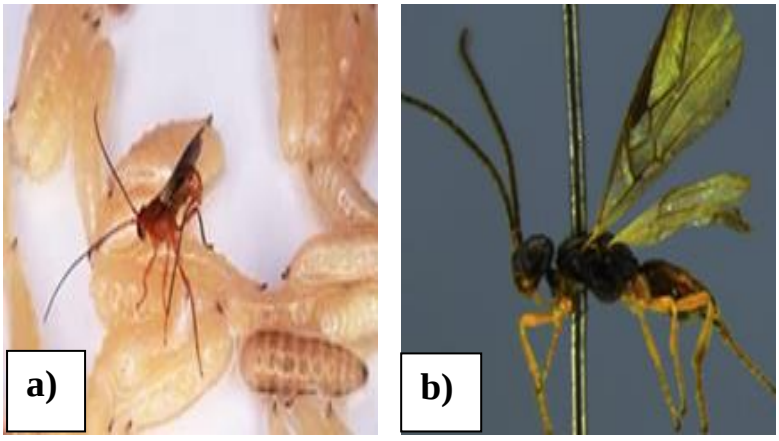

Figure 16. Morphology of Diachasmimorpha longicaudata: (a) Adult female ovipositing in host larvae (b) Lateral view of adult parasitoid (Vargas *et al*., 2015).

2.9 Factors that can affect dispersal of natural enemies

2.9.1 Establishment of natural enemies

The establishment of released parasitoids, such as *F. arisanus* and *D. longicaudata*, is significantly influenced by numerous factors that collectively enhance their chances of successful integration into new environments: plant diversity, competition with other natural enemies, parasitoid behavior, and physiological compatibility with local populations, host availability, and adaptability to environmental conditions (Kassie et al., 2020).

Most studies provide essential experimental data on the establishment success rates of parasitoids such as *F. arisanus* and *D. longicaudata*. Effective monitoring methods are crucial for evaluating the establishment of parasitoids after their release into the environment (Vargas et al., 2015). These methods help researchers gather important data on various aspects of parasitoid populations, including their dynamics, dispersal patterns, and rates of parasitism over time. Field surveys are one of the primary tools used in this monitoring process (Ndlela et al., 2022). For example, studies utilizing mark-release-recapture (MRR) techniques have shown promise in tracking the dispersal of parasitoids, allowing researchers to assess their movement and establishment in the field (Ekesi et al., 2016).

Landscape structure and agricultural practices play an important role in determining the establishment success of natural enemies such as *F. arisanus* and *D. longicaudata*. Heterogeneous agro-ecosystems with mixed cropping systems, presence of alternative hosts, and semi-natural vegetation provide refuges, food resources, and stable microhabitats that enhance parasitoid survival after release (Landis et al., 2000). These conditions increase the likelihood of successful colonization and long-term persistence of parasitoid populations in new environments (Ekesi et al., 2016). In contrast, highly simplified monoculture systems reduce habitat suitability by limiting resources and host diversity, which can negatively affect establishment success (Kassie et al., 2020). Furthermore, intensive orchard management practices, particularly frequent pesticide applications, may reduce parasitoid survival and disrupt early population establishment by interfering with host parasitoid interactions (Ndlela et al., 2022).

Despite establishment, the dispersal is a critical aspect of the ecology of natural enemies, such as *F. arisanus* and *D. longicaudata*, particularly in the context of biological control of pest populations (Agboka et al., 2024). Several factors influence the dispersal capabilities of parasitoid wasps, including the release point, movement patterns, final dispersal locations,

and adaptations that facilitate long-distance dispersal, environmental conditions, such as wind speed, relative humidity, and temperature, significantly affect parasitoid movement (Avraham et al., 2024).

The initial release point of natural enemies plays a crucial role in determining their subsequent dispersal patterns, habitat quality significantly impacts the survival and establishment of released parasitoids, and environments with abundant host availability, suitable microclimates, and minimal competition enhance their chances of settling (Kassie et al., 2020). Releasing natural enemies close to established pest populations increases the likelihood of successful parasitism. For example, if *F. arisanus* is released near areas heavily infested with *B. dorsalis*, the chances of their dispersal toward these hosts increase (Agboka et al., 2024). The timing of the release in relation to pest life cycles and coordinating releases with peak pest populations can enhance the effectiveness of natural enemies (Ndlela et al., 2022).

Post-release movement of natural enemies is also influenced by olfactory cues, both *F. arisanus* and *D. longicaudata* utilize olfactory cues to locate hosts, enabling them to detect volatile compounds emitted by infested fruits and move toward areas with higher host densities (Avraham et al., 2024). Environmental conditions, such as wind speed, humidity, and temperature, can significantly affect the movement of natural enemies. For instance, high humidity levels can enhance the survival and activity of natural enemies, while moderate temperatures can optimize their metabolic rates, leading to increased movement and foraging efficiency (Vargas et al., 2015).These parasitoids will eventually settle in areas with abundant hosts, leading them to regions where pest populations are concentrated, they may also seek microhabitats that offer protection and resources, such as shaded areas or regions with high plant diversity (Muhammad et al., 2023).

Landscape structure also strongly influences the dispersal of natural enemies. Habitat connectivity provided by mixed cropping systems and semi-natural vegetation facilitates movement of parasitoids across agricultural landscapes, allowing them to locate new host patches efficiently (Tscharntke et al., 2016). In contrast, fragmented or simplified monocultures can restrict movement due to limited ecological corridors and reduced host distribution (Vargas et al., 2015).

3.0 MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted in two provinces of Mozambique: Maputo and Cabo delgado provinces (Figure 17). The sites were selected based on the importance of fruit production and the presence of fruit flies and their natural enemies, allowing the assessment of suppression treatments and exotic parasitoid activity.

The first study site was located in Boane District, within Maputo Province in southern Mozambique (Latitude: -26.0958, Longitude: 32.39119), and fieldwork was carried out from April to August 2025. This site was selected basing on the presence of continuous fruit availability made the area suitable for evaluating fruit fly suppression treatments using bait stations and the male annihilation technique (MAT) (José et al., 2013).

Boane District lies about 45 km from Maputo and is bordered by Moamba District to the north, Namaacha District to the west and southwest, Matutuine District to the south and southeast, and Matola to the east. The district had good agricultural infrastructure and transport connections, including the Maputo corridor road, which facilitated agricultural activities and orchard management.

The second part of the study, related to the dispersal of introduced parasitoids, was conducted in Cabo Delgado Province in northern Mozambique, specifically in Pemba District (Latitude: -13.0701, Longitude: 40.40936). Fieldwork in this region was carried out from September to December 2025, corresponding to the mango fruiting period, which is favorable for fruit fly development and parasitoid activity. The area experiences a tropical coastal climate, with annual temperatures generally ranging between 22 °C and 34 °C. Cooler conditions occurred between June and August, while the warmest months were typically December and January (Cugala, 2011).

Agriculture in Pemba District included the production of mango, citrus, cashew, and other fruit crops that serve as hosts for fruit flies. Continuous availability of host plants supported both pest populations and naturally occurring parasitoids, making the region appropriate for studying parasitoid dispersal and parasitism rates (Mwatawala et al., 2009).

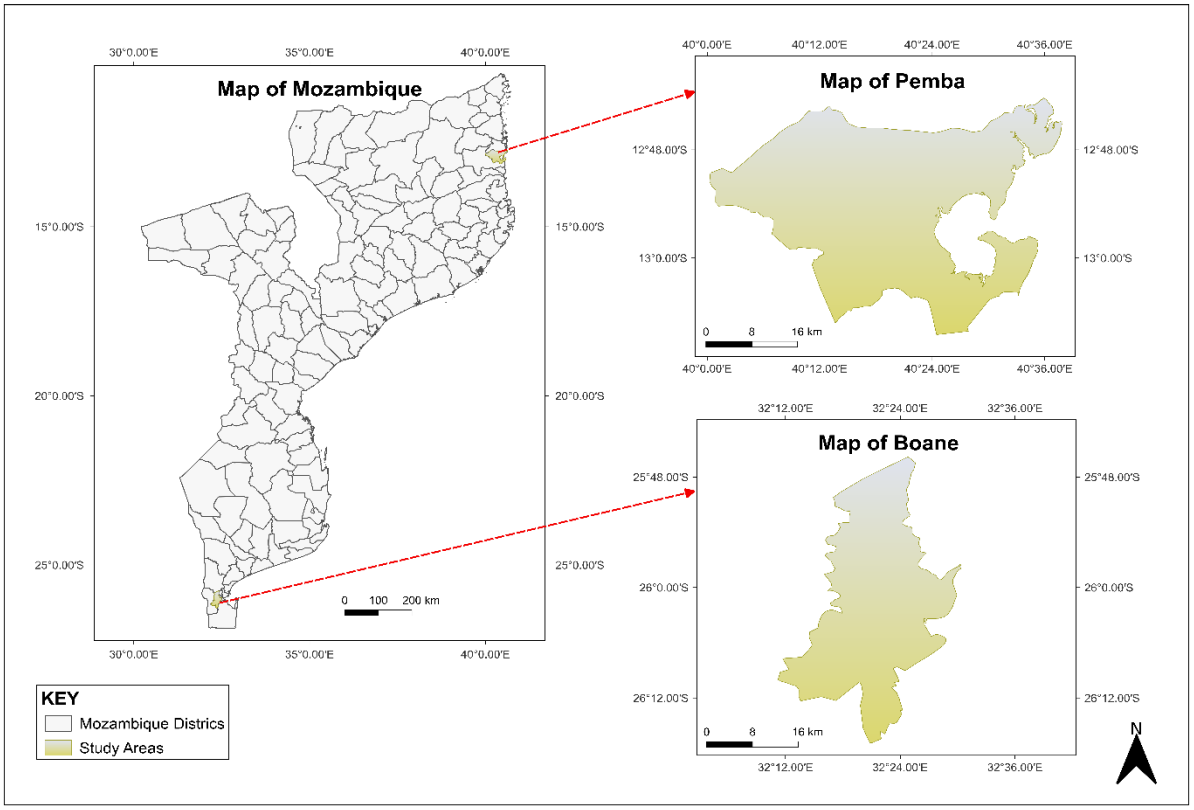

Figure 17. Location of the study areas (Map created by author using QGIS, 2025)

3.2 Sampling procedures

3.2.1 Effectiveness of M3 alone and M3 in combination with male annihilation technique in reducing fruit fly density in Boane District

Three orchards were selected in Boane District, and each orchard was assigned a specific treatment: a control or no treatment (26°03′10.11″ S, 32°19′21.87″ E; and 7 m a.s.l), M3 alone (26°06′12.13″ S, 32°24′22.92″ E; and 24 m a.s.l), and M3 in combination with the Male Annihilation Technique (26°10′15.13″ S, 32°24′24.95″ E; and 25 m a.s.l).

Each experimental plot measured approximately one hectare, ensuring uniformity and comparability across treatments. The installation of devices followed recommended guidelines, and replacements were carried out according to standard protocols, whereby M3 bait stations were replaced every three months and MAT devices every one month (Niassy et al. 2022). Replication was achieved through repeated weekly observations conducted throughout the study period, where each sampling event constituted a temporal replicate for all treatments and allowed for reliable comparison of treatment effects under field conditions (Vargas et al., 2015).

3.2.1.1. Deployment of treatments

a) No control

The control treatment consisted of orchard without applying any suppression method. This setup provided baseline data on natural fruit fly population dynamics for comparison with treated plots (FAO/IAEA, 2018).

b) M3 alone

The second treatment consisted of orchards where M3 bait stations were applied as the sole suppression method. They were hung within the tree canopy at approximately 1.5–2 m above ground level (Figure 18). This placement was chosen to target the main flight zone of adult fruit flies, enhance bait visibility and accessibility, and minimize interference from ground-level disturbances (Vargas et al., 2015).

Figure 18. Preparation and installation of M3 bait stations: (a) M3 bait station ready for use (b) Installing M3 bait stations in fruit trees.

The deployment density was 330 M3 bait stations with in hectare corresponds to a high-density attract-and-kill strategy, typically equivalent to approximately one bait station every 4–6 meters, ensuring effective coverage of the orchard to maximize contact with foraging fruit flies, particularly females seeking protein sources for egg development and effective attraction radius of each station (Ndlela et al., 2022). In this treatment, M3 bait stations were uniformly distributed across the entire plot, including the central area.

c) M3+ MAT

The third treatment involved the integration of M3 bait stations with the Male Annihilation Technique (MAT) within a one-hectare plot. In this treatment, M3 bait stations were strategically placed mainly inside the orchard (central zone) as described in A.2, while MAT devices were distributed in the perimeter of the plot (Ndlela et al. 2020). Male Annihilation Technique (MAT) devices were deployed in the study area to specifically target male fruit flies (Figure 19).

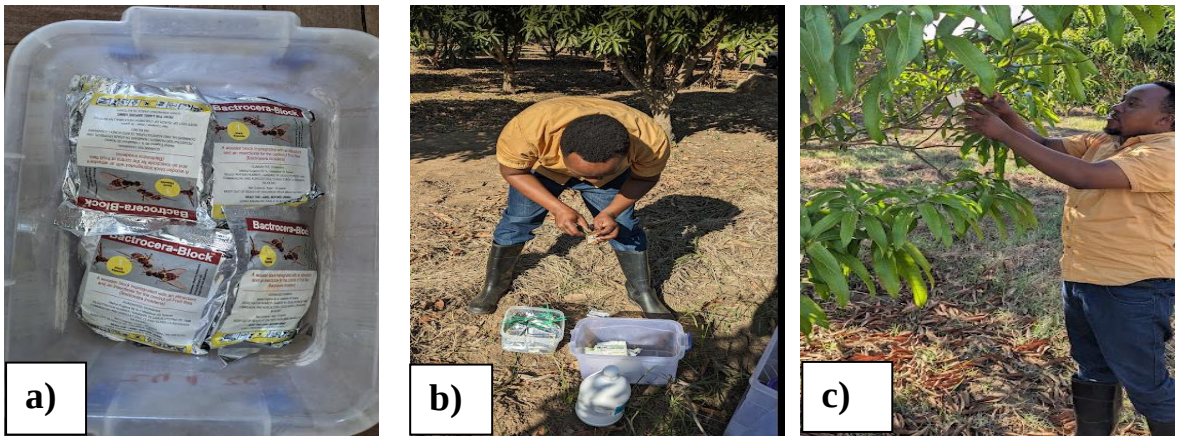

Figure 19. Preparation and installation of Male Annihilation Technique (MAT) devices: (a) MAT devices packaged; (b) Preparing and loading of MAT devices; (c) MAT devices placement within the orchard among fruit trees.

About 320 M3 bait stations per hectare were deployed in the central area of the orchard, and 24 MAT devices per hectare were placed along the perimeter and this arrangement provided high-density protein baiting targeting female fruit flies and low-density perimeter MAT deployment targeting males (Manrakhan et al., 2013). MAT devices were located at the edge of the orchard, approximately 15 meters from the nearest M3 bait stations (Fezza et al., 2024). This arrangement was based on the functional differences between the two techniques: MAT devices have a long-range attraction capacity, typically reaching distances of 50–100 meters or more, allowing them to attract male fruit flies from outside and across the plot (FAO/IAEA, 2018). In contrast, M3 bait stations act at short range, targeting flies already present within the orchard. Therefore, placing M3 bait stations inside the plot enhanced local suppression, while MAT provided broader attraction and population reduction at a larger spatial scale (Ndlela et al., 2020).

In all the 3 orchards, fruit flies were monitored using torula yeast traps uniformly used across all treatments. Two monitoring traps were placed per treatment (FAO/IAEA, 2018).

3.2.1.2. Preparation and installation of torula yeast for fruit flies population monitoring

Five Torula yeast tablets were dissolved in 500 mL of clean water prior to field installation to prepare the protein bait solution, this preparation was done in advance to ensure complete dissolution of the tablets and uniform distribution of the bait in the traps and prepared solution was then poured into the traps before they were installed in the study area (FAO/IAEA, 2018). The traps were positioned within the tree canopy at a height of approximately 1.5 to 2 meters above ground level, preferably in shaded areas to enhance trap efficiency (Figure 20) (FAO, 2012).

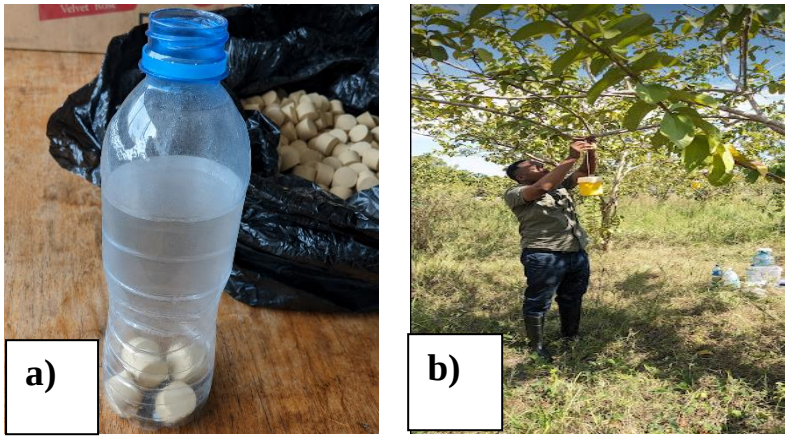

Figure 20. Preparation and Installation of a torula yeast trap for monitoring fruit fly population.

In each orchard, all traps baited with torula attractants were inspected on a weekly basis to ensure consistent monitoring of fruit fly populations (Ekesi et al., 2016. During each inspection, captured insects were carefully removed from the traps by separating them from the liquid attractant using a sieve, after which the suspension was poured and the specimens were rinsed with clean water (FAO/IAEA, 2018). The insects were then preserved in plastic vials containing 70% alcohol and appropriately labeled according (Figure 21). After sample collection, traps were thoroughly cleaned with water and freshly re-baited (Santos et al., 2022). All collected insects were transported at the Laboratory of Entomology at the Faculty of Agronomy and Forestry Engineering for further processing.

Figure 21. Trap monitoring and attractant replacement in the orchard: (a) collection of captured insects; (b) preserving captured insects in alcohol; (c) replacement of old torula attractants with fresh ones.

In the laboratory, fruit fly specimens were sorted from other insects based on their morphological features, particularly wing venation patterns, which allow clear identification within the sample. The selected fruit fly individuals were then cleaned, counted, and systematically recorded for subsequent data analysis (FAO/IAEA, 2018).

3.2.2 Determination of infestation levels of fruit flies and parasitism rates in different hosts in Maputo and Cabo Delgado provinces

Fruit fly host fruits were collected both from the ground and directly from trees in the two study provinces of Maputo and Cabo Delgado. In Maputo, fruit collection was conducted from April to August 2025, corresponding to the main fruiting and harvesting period of citrus, guava, June plum, and tropical almond. In contrast, in Cabo Delgado, fruit collection took place from September to December 2025, according with the peak fruiting and harvesting period of the mango season.

In Maputo province, sampling was conducted at Boane district across three sites, namely Quinta das Abelhas, Umbeluzi, and Picoco, which were selected based on fruit availability. The sites were separated by measurable distances to ensure representative sampling across the landscape, with approximately 4 km between Quinta das Abelhas and Umbeluzi, and 6 km between Umbeluzi and Picoco.

At Boane district, the following hosts were collected per location:

- Quinta das Abelhas - *Spondias dulcis* (june plum);
- Umbeluzi - *Psidium guajava* (guava) and *Terminalia catappa* (tropical almond);
- Picoco - *Citrus sinensis* (citrus).

In Cabo delgado, sampling was conducted from September to December 2025, according to the mango fruiting season. Mango fruits were collected across spatially defined zones, namely in Metuge district (locality of Mieze and Komakoma), Ancuabe district (Metoro and Silva Macua localities) and Chiúre district, representing an increasing distance gradient from the reference site. This spatial arrangement was designed to capture variation in fruit fly infestation across ecological gradients and potential source populations (at Mieze), the initial release site of the exotic parasitoids (released in 2010 and 2013). All sampling locations were georeferenced using GPS to ensure accurate positioning of sites and to avoid spatial bias or location misclassification during data collection (FAO/IAEA, 2018)

The collected fruits were placed in plastic containers (bowls), which were covered with lids fitted with a fine mesh to prevent parasitoids from escaping and transported at the Laboratory of Entomology, Faculty of Agronomy and Forestry Engineering where the fruits were incubated for the emergence of fruit flies and parasitoids (Figure 22).

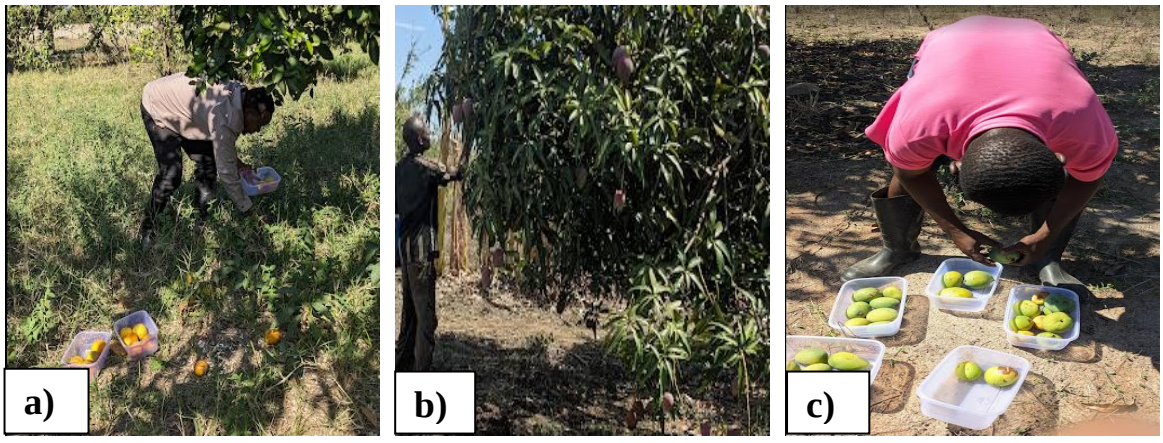

Figure 22. Fruit sampling and handling: (a) collection from the ground; (b) collection from the tree; (c) placement into containers for rearing.

In the laboratory, all collected fruits were counted, weighed, and examined for signs of damage. A fruit was considered damaged by fruit flies if it showed puncture wounds, discoloration, signs of decay, or the presence of larvae. After inspection, the fruits were placed individually in appropriate plastic containers and incubated for a period of 15–45 days

to allow larvae to pupate and the incubation setup consisted of two stacked plastic containers. The upper container had perforations at the base to allow larvae to exit, while the lower container contained sterilized sand to facilitate pupation (Vargas et al., 2015). The fruits were placed in the upper container (Figure 23). The containers were checked daily, and pupae were collected by sifting the sand (Ekesi & Billah, 2007).

Figure 23. Incubation of infested fruits and pupae collection: (a) fruits incubated in containers; (b) daily pupae collection by sifting sand.

The collected pupae were counted and placed in Petri dishes containing moistened filter paper. Subsequently, the pupae were transferred to emergence cages for the development and emergence of adult fruit flies and potential parasitoids (Ekesi & Billah, 2007). Emerging adults were maintained in the cages and fed with honey and water soaked in cotton. They were kept for 3 to 5 days to allow full development of morphological characteristics necessary for identification (Vargas et al., 2015). A few drops of ethyl acetate were applied to immobilize the fruit flies. At the end, all incubated fruits were checked again to recover any remaining larvae or pupae (Figure 24) (Niassy et al. 2022).

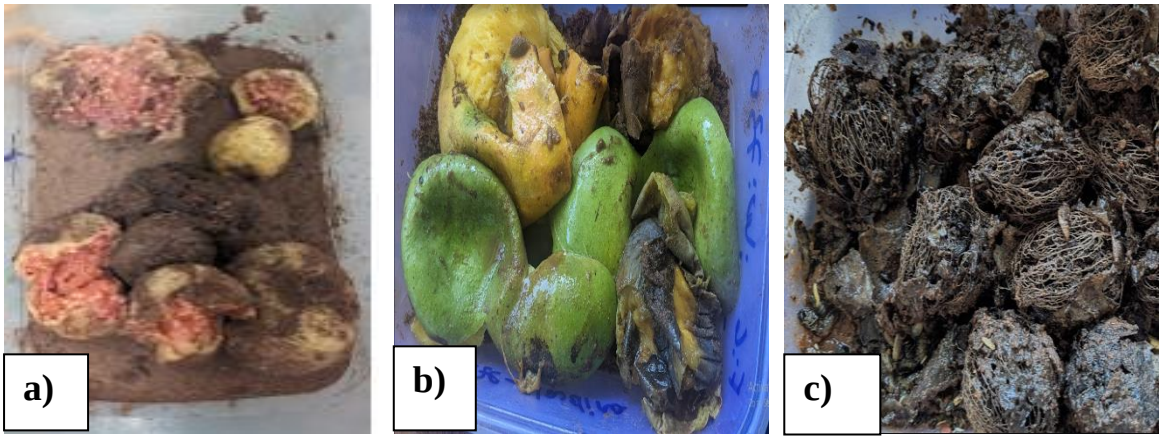

Figure 24. Dissection of fruits for recovery of larvae and pupae: (a) guava; (b) mango; (c) June plum.

Parasitoid adults were separated from fruit flies. All emerged adults (fruit flies and parasitoids) were counted and identified to species level at the Laboratory of Entomology, Faculty of Agronomy and Forestry Engineering.

Following emergence, parasitoids were separated from fruit flies and all adults were counted. Specimens were identified to species level using morphological characters and relevant taxonomic keys under a stereomicroscope at the Laboratory of Entomology, Faculty of Agronomy and Forestry Engineering. Data on the number of fruit flies and parasitoids emerging from each fruit sample were recorded to estimate infestation indices and parasitism rates. Representative specimens of each species were preserved as voucher material for reference.

3.2.3 Measurements of the introduced parasitoids dispersal distance in Cabo Delgado Province

The study area was divided into five sampling zones (as described in 3.2.2 section) based on increasing distance from the original parasitoid release site in Mieze (13°6′01.10″ S, 40°24′53.61″ E; 89 m a.s.l.) (Figure 18). Additional sampling locations were established in Komakoma (13°04′40.21″ S, 40°17′02.51″ E; 78 m a.s.l.), Metuge (12°48′42.23″ S, 40°12′04.15″ E; 18 m a.s.l.), Metoro (13°12′05.15″ S, 39°48′56.79″ E; 16 m a.s.l.), Silva Macua (12°36′15.27″ S, 39°48′34.58″ E; 12 m a.s.l.), and Chiure (13°24′04.16″ S, 39°36′35.34″ E; 10 m a.s.l.).

Based on their distance from the release site, the sampling locations were grouped into five zones: Zone 1 (Komakoma, 7.31 km), Zone 2 (Metuge, 21 km), Zone 3 (Metoro, 50 km), Zone 4 (Silva Macua, 74.5 km), and Zone 5 (Chiure, 110 km). Distances were calculated from GPS coordinates. This spatial arrangement facilitated the assessment of parasitoid dispersal along a gradient of increasing distance from the release site (Figure 25).

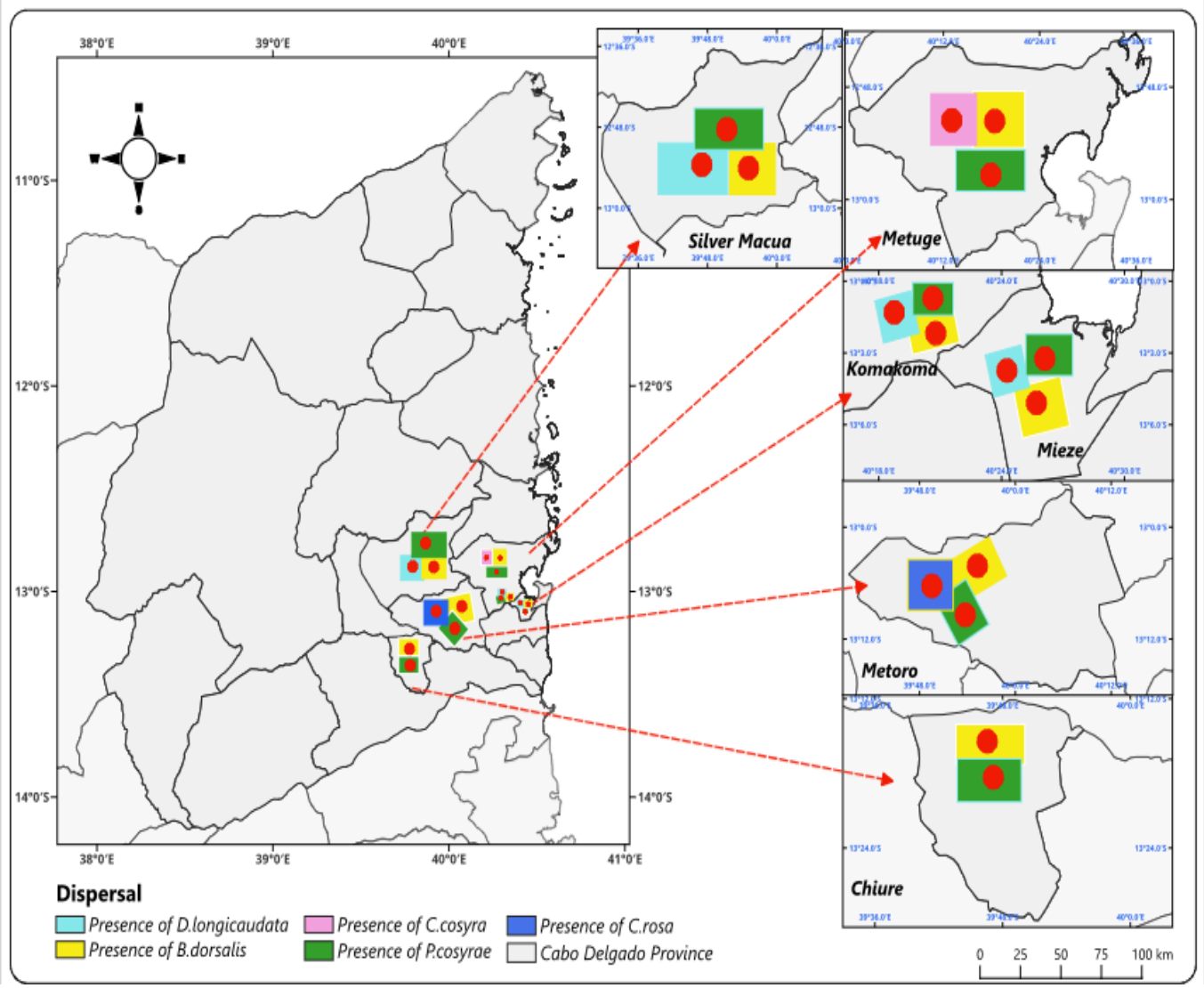

Figure 25. Map of the study area showing the parasitoid release site in Cabo delgado and sampling locations (Komakoma, Metuge, Metoro, Silva Macua, and Chiure) used to assess dispersal distance.

The dispersal of released parasitoids was measured using Global Positioning System (GPS) coordinates to determine the distance (D) from the release site to each sampling point where parasitoids were recovered. Dispersal distance was estimated using the Mean Dispersal Distance (MDD) as described by Ekesi et al., (2016).

3.3 Determined variables

3.3.1 Absolute abundance of fruit fly species

Absolute abundance of fruit fly species was determined by counting the total number of individuals of each species recovered from the samples in each treatment or sampling unit. This measure provides a direct estimate of species population size within each study site and allows comparison of overall fruit fly population levels across different locations and management treatments (Ekesi et al., 2009).

This measurement provided an indication of species dominance and variation in population distribution within the study sites. In addition, proportional representation of each species within the total captured collection was used to compare species contribution across treatments and fruit infestations.

The relative abundance was calculated by using the following formula:

$$\mathbf{RA}=\frac{ni}{N} \times 100 \qquad \text{(Formula 1)}$$

Where:

- **RA** = Relative abundance (%)
- $\mathbf{n_i}$ = Number of individuals of species *i*
- **N** = Total number of individuals of all fruit fly species collected

3.3.1 Flies per trap per day

Fruit fly density was expressed as the average number of flies caught per trap per day (FTD), which provided a reliable estimate of the adult population under different suppression treatments and the control, as described by (FAO/IAEA, 2018). The FTD value was calculated using the following formula:

$$\mathbf{FTD} = \frac{F}{T \times D} \qquad \text{(Formula 2)}$$

Where:

- FTD = Fruit fly Trap Density (number of flies per trap per day)
- F = Total number of adult flies captured in all traps during the sampling period

- T = Number of traps deployed in the orchard or treatment plot
- D = Number of days the traps were exposed in the field

3.3.2 Fruit flies infestation indices

Four infestation indices were used, namely: pupae per fruit, pupae per kilogram of fruit, adults per fruit, and adults per kilogram of fruit (Raga et al., 2017). These indices allowed comparison of infestation levels between hosts and locations. The indices were calculated using the following formulas:

i) Pupae Infestation Index per Fruit (Pupae/Fruit)

$$\textbf{Pupae per Fruit} = \frac{Total\ number\ of\ pupae\ harvested}{Total\ number\ of\ Fruits\ incubated} \quad \text{(Formula 3)}$$

ii) Pupae Infestation Index per Kilogram (Pupae/kg)

$$\textbf{Pupae per Kilogram} = \frac{Total\ number\ of\ pupae\ harvested}{Total\ weight\ \ of\ fruits\ incubated\ (kg)} \quad \text{(Formula 4)}$$

iii) Adult Infestation Index per Fruit (Adults/Fruit)

$$\textbf{Adults per Fruit} = \frac{Total\ number\ of\ adults\ emerged}{Total\ number\ of\ Fruits\ incubated} \quad \text{(Formula 5)}$$

iv) Adult Infestation Index per Kilogram (Adults/kg)

$$\textbf{Adults per Kilogram} = \frac{Total\ number\ of\ adults\ emerged}{Total\ weight\ of\ Fruits\ incubated} \quad \text{(Formula 6)}$$

3.3.3 Parasitism rate of the parasitoids of both native and exotic parasitoids

Parasitism rate was estimated based on the number of parasitoids that emerged from collected fruit samples relative to the total number of insects that emerged, including both fruit fly adults and parasitoids. This measure indicated the level at which parasitoids successfully developed within fruit fly hosts under field conditions.

A higher proportion of emerged parasitoids compared to fruit fly adults indicated effective parasitoid activity and suggested successful establishment and reproduction of parasitoids

within the ecosystem as described by Cugala et al. (2016). The parasitism rate was calculated using the following formula:

$$\mathbf{P} = \frac{\mathbf{T_P}}{\mathbf{T_m + T_p}} \times \mathbf{100} \qquad \text{(Formula 7)}$$

Where: T_p = Emerged parasitoid adults

T_m = Emerged fruit fly adults

P= Total of parasitoids

Parasitism rates were calculated based on the number of parasitized units in which parasitoids were recovered, rather than total fruit fly emergence, in order to precisely reflect host parasitoid interactions.

3.3.4 Parasitoid dispersal distance

Dispersal distance was estimated using the Mean Dispersal Distance (MDD) as described by Ekesi et al. (2016). The MDD was calculated as the weighted average of distances based on parasitoid recovery at different sampling sites relative to the original release point in Mieze. Maximum dispersal distance (Dmax) was determined as the farthest sampling location at which parasitoids were detected. Together, these parameters provided an assessment of the spatial distribution patterns and the potential long-term establishment of *F. arisanus* and *D. longicaudata* in Cabo delgado. Mean Dispersal Distance (MDD) was calculated using the following formula:

$$\mathbf{MDD} = \frac{\sum(\mathbf{d_i} \times \mathbf{n_{i,s}})}{\sum \mathbf{n_{i,s}}} \qquad \text{(Formula 8)}$$

Where by:

d_i = distance from the release point to sampling location i

ni,s = number of parasitoids of species s recorded at location i

s = parasitoid species (*F. arisanus* or *D. longicaudata*)

3.4 Data analysis

Descriptive statistics, including means and percentages, were used to summarize the different parameters. Fruit fly density (FTD) values were calculated for each treatment and sampling period, and summary statistics (mean ± standard error) were generated, data were analyzed using one-way ANOVA to evaluate the effects of M3 alone and M3 combined with MAT on fruit fly density, with Tukey's post-hoc test applied at the 5% significance level for mean separation.

Infestation indices (pupae per fruit, pupae per kilogram, adults per fruit, and adults per kilogram) were also computed for each host, followed by the calculation of means and associated standard errors, one-way ANOVA was also used; however, when assumptions of normality and homogeneity were violated, a non-parametric Kruskal–Wallis test was applied, followed by Dunn's test for multiple comparisons at the 5% significance level.

Parasitism rates were determined for each district and parasitoid species, and mean percentages were calculated.

Mean Dispersal Distance (MDD) and maximum dispersal distance (Dmax) were analyzed using descriptive statistics.

All laboratory data were entered into Microsoft Excel, and statistical analyses, including graphical outputs, were performed using R software (R version 2025), with statistical significance set at $p < 0.05$.

4.0 RESULTS

4.1 Effect of M3 alone and M3 in combination with the Male Annihilation Technique (MAT) on fruit fly density in Boane District

4.1.1. Absolute and relative abundance of the different fruit fly species

Data were collected over a total of twenty-three (23) weeks. Throughout this sampling duration, fruit flies were systematically trapped across the different treatments, allowing for a comprehensive assessment of species composition and abundance over time. Across the three treatments (control, M3 alone and M3+MAT), were trapped fruit flies belonging to the genera *Bactrocera*, *Dacus*, and *Ceratitis*. The following fruit fly species were recorded: *Bactrocera dorsalis*, *Dacus bivittatus*, *Dacus punctatifrons*, *Dacus vertebratus*, *Dacus pergularia*, *Dacus frontalis*, *Dacus ciliatus*, *Ceratitis capitata*, *Ceratitis cosyra*, *Ceratitis punctata*, *Ceratitis rosa*, and *Ceratitis quilicii* (Table 1). Overall, the highest number of individuals was captured in the control orchard (2,176 flies), followed by M3 alone (960 flies), while the lowest abundance was observed in the M3+MAT treatment (314 flies), indicating a strong reduction in fly catches when the combined treatment was applied (Table 1).

Across all treatments, *B. dorsalis* was consistently the dominant species, accounting for the largest proportion of captures in each treatment (64.25% in the control, 68.23% in M3 alone, and 64.33% in M3+MAT). *Dacus bivittatus* represented the second most abundant species, with 708 species corresponding to a relative abundance of (32.53%) in the control, 258 species corresponding to a relative abundance of 26.87% in M3 alone, and 101 species corresponding to a relative abundance of 32.16 % in M3+MAT, showing a clear decline under the combined treatment (Table 1). Other species of the genus *Dacus* were recorded in relatively low numbers across all treatments, with some species being completely absent in the M3+MAT treatment. Similarly, species of the genus *Ceratitis* such as *C. quilicii*, *C. rosa*, *C. capitata*, *C. cosyra*, and *C. punctata* were present in low abundance, with a marked reduction in captures under M3+MAT (Table 1).

Table 1. Absolute and relative abundance of the across treatments

Species	**TREATMENTS**						
	Control		**M3 Alone**		**M3+MAT**		**GRAND TOTAL**
	A/A	**R/A (%)**	**A/A**	**R/A (%)**	**A/A**	**R/A (%)**	
Bactrocera dorsalis	1398	64.25	655	68.23	202	64.33	2255
Dacus bivittatus	708	32.54	258	26.88	101	32.17	1067
Dacus puntactifrons	10	0.46	4	0.42	1	0.32	15
Dacus vertebratus	6	0.28	7	0.73	3	0.96	16
Dacus pergularia	4	0.18	5	0.52	0	0	9
Dacus frontalis	8	0.37	3	0.31	2	0.64	13
Dacus ciliatus	3	0.14	4	0.42	0	0	7
Ceratitis capitata	9	0.41	6	0.63	0	0	15
Ceratitis cosyra	3	0.14	1	0.10	0	0	4
Ceratitis punctata	2	0.09	0	0	0	0	2
Ceratitis rosa	5	0.23	3	0.31	1	0.32	9
Ceratitis quilicii	20	0.92	14	1.46	4	1.27	38
TOTAL	2176	100	960	100	314	100	3450

The mean fruit fly density (FTD) was further studied for the two most abundant fruit fly species, which differed significantly among treatments for both *B. dorsalis* and *D. bivittatus* (Table 2). For *B. dorsalis*, the control treatment recorded the highest FTD (8.68 ± 1.26), followed by M3 alone (4.07 ± 0.9), while the lowest value was observed under M3 bait stations and M3 with combination of MAT (1.25 ± 0.28). A similar pattern was observed for *D. bivittatus*, where the control treatment had the highest FTD (4.4 ± 1.04), followed by M3 alone (1.8 ± 0.65), and the lowest value under M3 bait stations and M3 with combination of MAT (0.63 ± 0.2).

Table 2. Mean Fruit Fly Density (FTD ± SE) of *Bactrocera dorsalis* and *Dacus bivittatus* across the treatments.

Treatments	**Fruit fly species**	
	B. dorsalis	*D. bivittatus*
Control	8.68 ± 1.26a	4.4±1.04a
M3 alone	4.07± 0.9b	1.8±0.65b
MAT and M3	1.25± 0.28b	0.63±0.2b
p -value	1.1e-06	0.001

*Means sharing the same letter within each column are not significantly different at $p < 0.05$.

Statistical comparisons using Tukey post-hoc test indicated that mean fruit fly densities differed significantly among treatments, where the control recorded significantly higher densities for both fruit fly species compared to M3 alone and M3+MAT treatments, which did not differ significantly from each other.

The graphical representation of the mean fruit fly density for *B. dorsalis* shows a clear variation among the treatments (Figure 26). The control treatment recorded the highest FTD, indicating a higher population of fruit flies in untreated plots. In contrast, both M3 alone and M3 in combination with MAT treatments revealed reduced FTD values, with MAT + M3 showing the lowest density among all treatments. This trend demonstrates a consistent decline in fruit fly population with the application of control measures.

Also, the difference between treatments is visually evident in the height of the bars (Figure 29), where M3 in combination with MAT showed a more definite reduction compared to M3 alone. The relatively smaller error bars associated with the treated groups suggest lower variability in fruit fly density under these treatments (Figure 26). Overall, the findings supports the effectiveness of the treatments in reducing *B. dorsalis* populations, particularly under the M3 in combination with MAT approach.

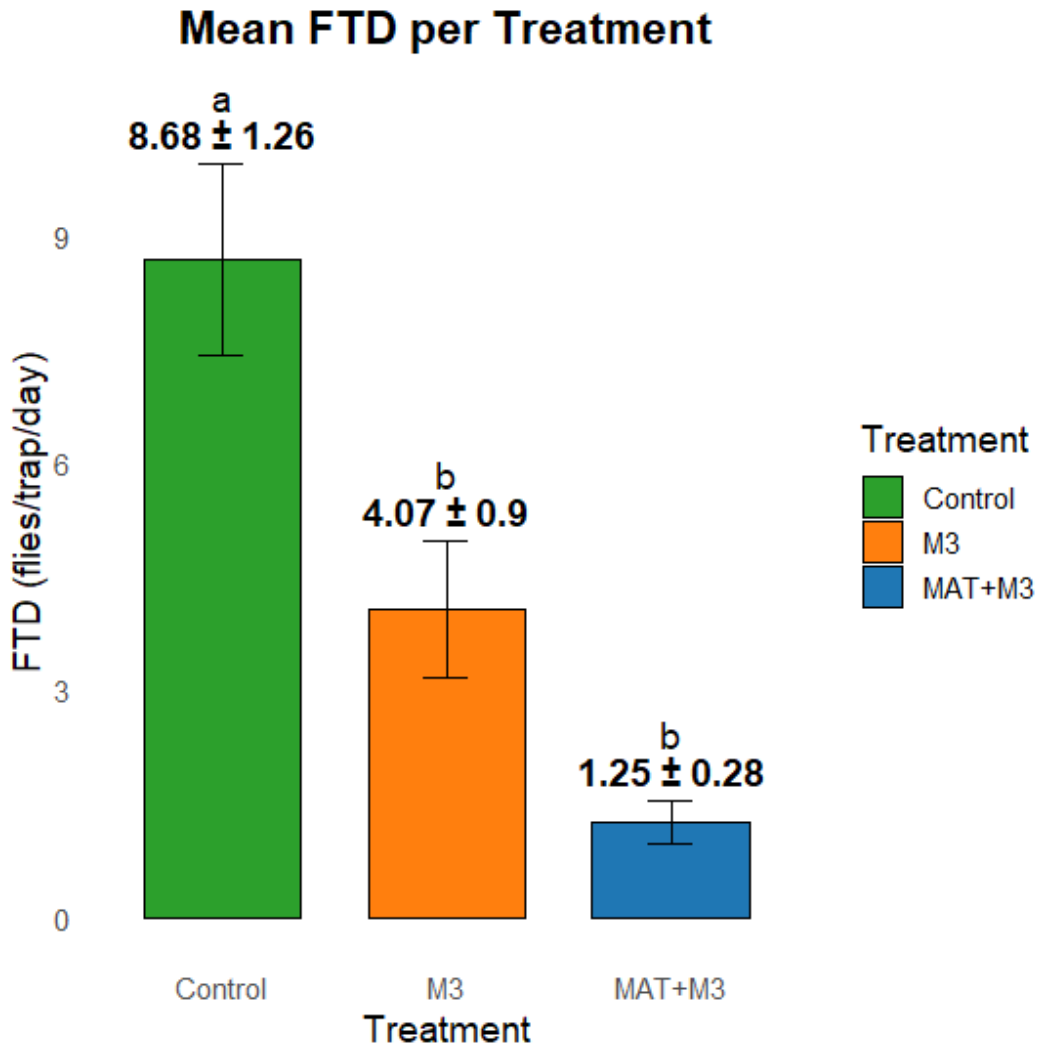

Figure 26. Mean Fruit fly Density per treatment for *Bactrocera dorsalis.* Bars sharing the same letter are not significantly different at $p < 0.05$.

The graph 30 illustrate the mean fruit fly density for *D. bivittatus* and similarly reveals distinct differences among treatments (Figure 27). The control treatment shows the highest FTD, while M3 alone and M3 in combination with MAT treatments demonstrate lower values. The M3 in combination with MAT treatment recorded the lowest fruit fly density, indicating a substantial reduction in population compared to the untreated control. In addition, the graphical pattern highlights a decrease in fruit fly density from control to M3 alone and further to the M3 in combination with MAT. Although some variability is observed, as indicated by the error bars, the overall trend remains consistent. This visual representation supports the numerical results and confirms that the applied treatments were effective in suppressing *D. bivittatus* populations.

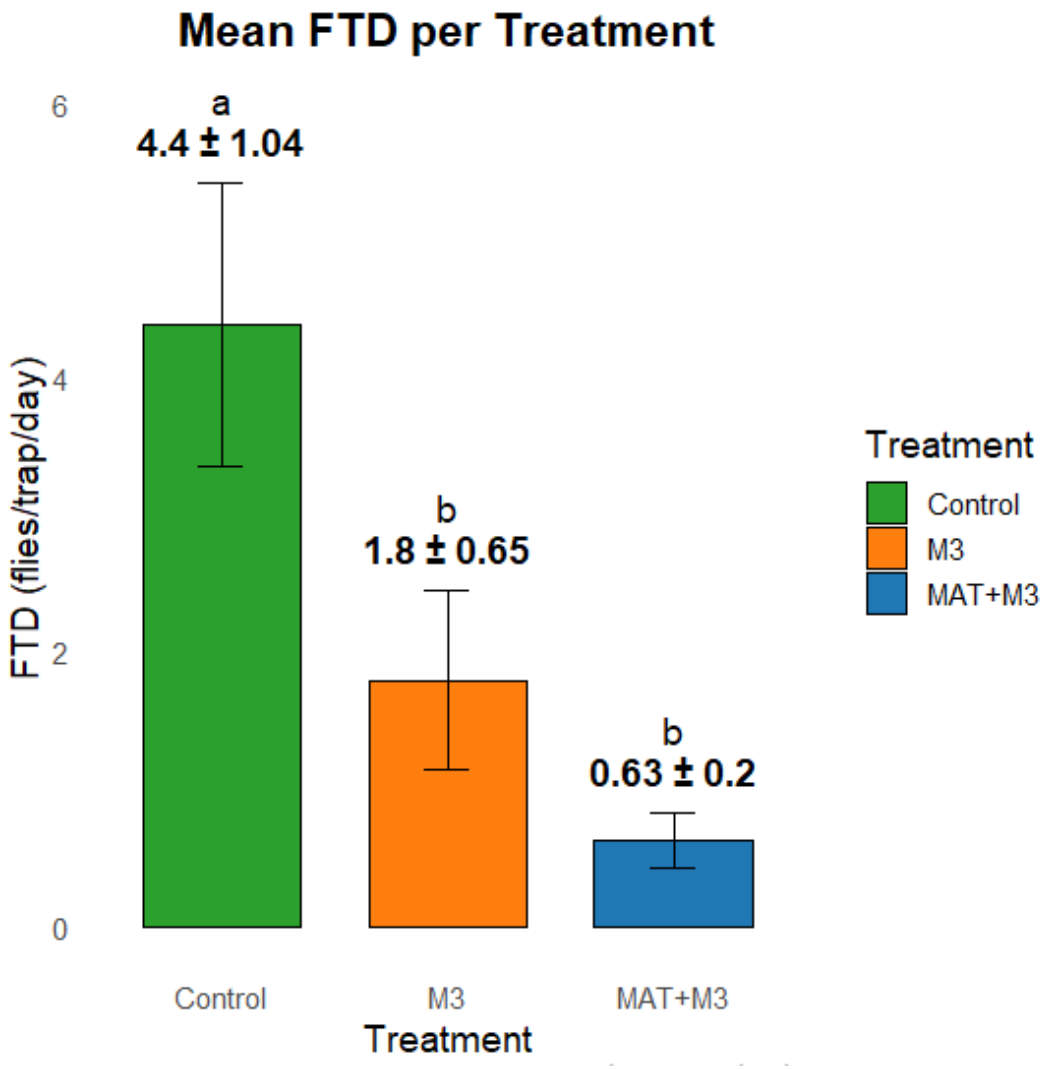

Figure 27. Mean Fruit fly Density per treatment for *Dacus bivittatus*. Bars sharing the same letter are not significantly different at $p < 0.05$.

4.2 Fruit flies infestation indices and parasitism rates

4.2.1 Infestation levels of *Bactrocera dorsalis* in different host fruits

Bactrocera dorsalis was selected as the target species for the calculation of infestation indices due to its marked dominance across all fruit types studied. At the genus level, *Bactrocera* accounted for 12,498 individuals, representing 99.89% of the total fruit flies collected, while *Ceratitis* species were recorded at a very low abundance of only 13 individuals (0.09%). The genus *Ceratitis* was indicated by *C. capitata*, *C. rosa*, and *C. cosyra*, all occurring at negligible levels (Table 3). Similarly, across guava, mango, citrus, tropical fruits, and june plum, *B. dorsalis* consistently represented nearly all captured fruit

flies (Approximately 99.9%), whereas *Ceratitis* species occurred in very low proportions. Therefore, focusing on *B. dorsalis* provides a reliable representation of the principal fruit fly species responsible for infestation in the study area.

Table 3. Absolute and relative abundance of fruit fly species across different fruits.

Locations	**Sites**	**Fruit types**	**Species**	**Absolute abundances (N)**	**Relative abundances (%)**
Maputo province	Umbeluzi	Guava	*B.dorsalis*	9215	99.94
			C.capitata	2	0.02
			C. rosa	4	0.04
		Total abundance on guava		9221	100
		Tropical almond	*B. dorsalis*	2705	99.92
			C.capitata	1	0.04
			C. rosa	1	0.04
		Total abundance on tropical almond		2707	100
	Picoco	Citrus	*B. dorsalis*	182	99.45
			C. rosa	1	0.55
		Total abundance on citrus		183	100
	Quinta des Abelhas	June plum	*B. dorsalis*	29	100
Cabo Delgado province	Metuge	Mango	*B. dorsalis*	25	6.74
			C.cosyra	2	0.54
	Silva Macua	Mango		20	2.7
	Chiure	Mango		89	24
	Metoro	Mango	*B. dorsalis*	84	25.33
			C. rosa	2	0.54
	Mieze	Mango		57	15.36
	Komakoma	Mango		92	24.79
		Total abundance on mango		371	100

A total of approximately 182 kg of fruits were collected across all sampling sites (Table 4). The majority of samples originated from Maputo province (2080 fruits), which also recorded the highest number of pupae (15,684) and adult fruit flies (12,131), compared to Cabo Delgado province with only 646 pupae and 367 adults. Among fruit types, guava showed the highest infestation levels, yielding 12,001 pupae and 9,215 adults, followed by tropical almond with 3,371 pupae and 2,705 adults. In contrast, citrus and June plum recorded the lowest recoveries. Mango fruits, collected mainly from Cabo Delgado, exhibited moderate infestation levels, with 646 pupae and 367 adult fruit flies. Additionally, within Maputo province, Umbeluzi site contributed the largest proportion of pupae and adults.

Table 4. Summary of total fruits, total weight, total pupae and fruit flies adults recovered per fruit.

Locations	**Sites**	**Fruits types**	**Total Fruits**	**Total weights(kg)**	**Total pupae**	**Total adults**
Maputo province	Quinta des abelhas	June plum	130	6.312	72	29
	Umbeluzi	Guava	1000	58.908	12001	9215
		Tropical Almond	350	9.386	3371	2705
	Picoco	Citrus	600	76.7	240	182
Totals in Maputo province			2080	151.306	15684	12131
Cabo delgado province	Metuge	Mango	48	5.067	63	25
	Silva Macua	Mango	48	4.437	45	20
	Chiure	Mango	36	4.246	120	89
	Metoro	Mango	54	4.672	159	84
	Mieze	Mango	36	4.412	94	57
	Komakoma	Mango	60	7.745	165	92
Totals in Cabo delgado province			282	30.579	646	367

Infestation indices differed markedly both among fruit types and between the two study locations (Table 5). Overall, Maputo province recorded substantially higher infestation levels across all evaluated hosts compared to Cabo Delgado province, where only mango was assessed and showed comparatively low infestation values.Within Maputo, guava and tropical almond consistently exhibited the highest infestation levels for all parameters. Guava recorded 12.00 ± 0.76 pupae per fruit and 198 ± 11.3 pupae per kg, while tropical almond showed even higher infestation intensity in weight-based indices (353 ± 35.0 pupae per kg and 283 ± 30.9 adults per kg). These two fruit types formed a statistically similar high-infestation group ($p < 0.001$). Citrus and June plum, in contrast, presented significantly lower infestation indices, with values close to zero for most parameters, indicating their relatively low suitability for fruit fly development.

Mango from Cabo Delgado exhibited intermediate but generally low infestation levels (2.29 ± 0.66 pupae per fruit and 23.6 ± 6.48 pupae per kg), significantly lower than guava and tropical almond in Maputo ($p < 0.001$), but higher than citrus and June plum for some parameters. Overall, the results demonstrate strong and highly significant effects of both fruit type and location on fruit fly infestation ($p < 0.001$), with Maputo's fruit hosts particularly guava and tropical almond supporting markedly higher pest development compared to mango in Cabo Delgado

Table 5. Mean (± SE) of pupae and adult infestation indices per fruit and per kg across fruits

Locations	**Fruit type**	**Pupae per fruits**	**Pupae per kg**	**Adults per fruits**	**Adults per kg**
Maputo province	Citrus	0.40 ± 0.10b	3.20 ± 0.85b	0.30 ± 0.08b	2.44 ± 0.70b
	Guava	12.00 ± 0.76a	198 ± 11.3a	9.22 ± 0.65a	152 ± 9.50a
	June plum	0.55 ± 0.16b	11.2 ± 3.34b	0.22 ± 0.10b	4.63 ± 2.16b
	Tropical almond	9.63 ± 1.00a	353 ± 35.0a	7.73 ± 0.88a	283 ± 30.9a
p -value		<0.001	<0.001	<0.001	<0.001
Cabo Delgado	Mango	2.29 ± 0.66b	23.6 ± 6.48b	1.30 ± 0.36b	13.5 ± 3.50b
p -value		<0.001	<0.001	<0.001	<0.001

*Means followed by the same letters in the column are not significantly different at $p<0.05$.

4.2.2 Parasitism rates of native and introduced parasitoids

The recovered parasitoid species, their host fruit associations, sampling locations, and abundance are summarized in Table 6. Overall, two parasitoid species were recorded across the study areas: the native *Psyttalia cosyrae* and the exotic *Diachasmimorpha longicaudata* (Figure 28) with a clear difference in species composition between locations.

In Maputo province only the native *P. cosyrae* was recovered, and it was associated with all sampled fruit types. Its abundance was strongly host-dependent, with the highest emergence recorded in Umbeluzi site in guava (1243 individuals) followed by tropical almond (147 individuals), indicating that these fruit types supported greater parasitoid development in this location.

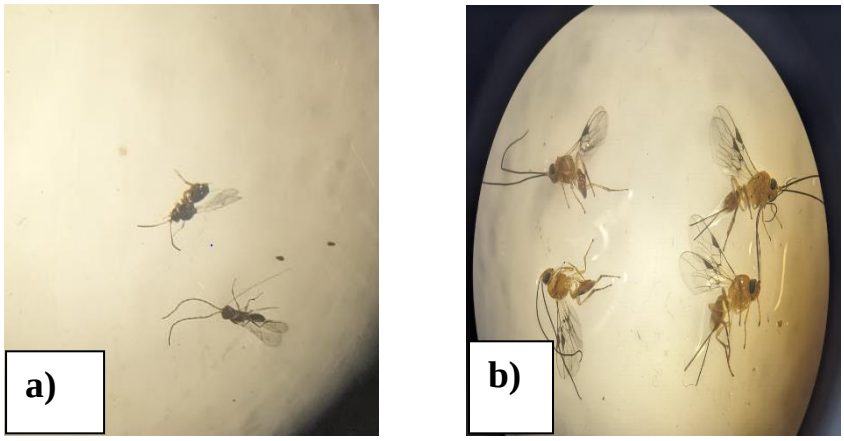

Figure 28. Emerged parasitoids: a) *Psyttalia cosyrae*; b) *Diachasmimorpha longicaudata*

In contrast, Cabo Delgado province showed a more diverse parasitoid collection, where both *P. cosyrae* and *D. longicaudata* were recovered from mango fruits. The native *P. cosyrae* was present across all sampled sites, with the highest abundance recorded in Metuge (7), followed by Chiure (5), Metoro (4), and the lowest values in Mieze and Komakoma (3 each) and Silva Macua (2). The exotic *D. longicaudata* was more restricted in distribution, being recorded only in Mieze (4), Komakoma (5) and Silva Macua (1).

Overall, these results indicate a strong spatial contrast in parasitoid community structure, with Maputo dominated exclusively by the native parasitoid and higher abundance linked to specific fruit hosts, while Cabo delgado exhibited lower but more diversified parasitoid presence, including the introduction and establishment of the exotic *D. longicaudata* in limited sites.

Table 6. Absolute and Relative abundance of parasitoid species across fruit types and location

Location	Site	Fruit type	Parasitoids species	Absolute abundance
Maputo province	Umbeluzi	Guava	*P. cosyrae*	1243
		Tripical almond	*P. cosyrae*	147
	Picoco	Citrus	*P. cosyrae*	28
	Quinta das abelhas	June plum	*P. cosyrae*	3
Cabo delgado province	Metuge	Mango	*P. cosyrae*	7
	Silva Macua	Mango	*P. cosyrae*	2
		Mango	*D. longicaudata*	1
	Chiure	Mango	*P. cosyrae*	5
	Metoro	Mango	*P. cosyrae*	4
	Mieze	Mango	*P. cosyrae*	3
		Mango	*D. longicaudata*	4
	Komakoma	Mango	*P. cosyrae*	3
		Mango	*D. longicaudata*	5

Parasitism rates varied markedly across fruit types, sampling locations, parasitoid species, and sites (Table 7). Overall, clear spatial and host-associated differences were observed between Maputo province (Boane area) and Cabo Delgado province, indicating strong ecological variation in parasitoid performance.

In Maputo province, parasitism was exclusively attributed to the native *Psyttalia cosyrae*, which was recovered from all sampled fruit types. The highest parasitism rates were recorded in citrus (13.33%), followed by guava (11.88%), June plum (9.37%), and tropical almond (5.15%). These results show that parasitism in Maputo was generally moderate, with citrus and guava acting as the most important hosts for parasitoid activity, while tropical almond supported comparatively lower parasitism levels despite high infestation in earlier results

The parasitism rates recorded across Cabo Delgado province showed noticeable variation among the six sampling locations. Overall, parasitism ranged from 3.16% to 10.93%,

indicating generally low to moderate parasitoid activity in the study area. Among all sites, Metuge recorded the highest parasitism rate (10.93%), suggesting relatively strong parasitoid effectiveness. In contrast, Komakoma showed the lowest rate (3.16%), despite having a high number of samples. Other locations such as Chiure (5.34%), Metoro (4.08%), and Mieze (5%) exhibited moderate parasitism levels.The species *P. cosyrae* was consistently present across all sites and constituted the majority of recovered parasitoids. On the other hand, *D. longicaudata* was less widespread but showed relatively higher parasitism in specific locations such as Mieze (6.55%**)** and Komakoma (5.15%), where it performed better than *P. cosyrae*.

In general, higher host sample numbers did not necessarily correspond to higher parasitism rates, as observed in Chiure, Metoro, and Komakoma. This suggests that parasitoid effectiveness varies by location and is not solely dependent on host availability. Overall, the results indicate that parasitoid activity in Cabo Delgado is present but unevenly distributed, with localized areas of relatively higher effectiveness.

Table 7. Parasitism rates associated with different fruit types

Locations	**Sites**	**Fruit type**	**Parasitoid**	**Total fruit flies**	**Total parasitoids**	**Parasitism rate (%)**
Maputo province	Quinta des abelhas	June plum	*P. cosyrae*	29	3	9.37
	Umbeluzi	Guava	*P. cosyrae*	9215	1243	11.88
		Tropical almod	*P. cosyrae*	2705	147	5.15
	Picoco	Citrus	*P. cosyrae*	182	28	13.33
Cabo delgado province	Metuge	Mango	*P. cosyrae*	25	7	10.93
	Silva Macua	Mango	*P. cosyrae*	20	2	9.09
			D. longicaudata		1	4.76
	Chiure	Mango	*P. cosyrae*	89	5	5.34
	Metoro	Mango	*P. cosyrae*	84	4	4.08
	Mieze	Mango	*P. cosyrae*	57	3	5
			D. longicaudata		4	6.55
	Komakoma	Mango	*P. cosyrae*	92	3	3.16
			D. longicaudata		5	5.15

4.3 Exotic parasitoid dispersal distance in Cabo delgado Province

The distribution of the exotic parasitoid Diachasmimorpha longicaudata across sampling locations in Cabo Delgado showed clear variation in relation to distance from the presumed release site (Table 8). A total of 10 individuals were recovered across all surveyed sites, with higher abundance recorded at locations closer to the release area.

Specifically, Mieze (0 km from the release point) recorded the highest abundance with 4 parasitoids, followed by Komakoma (7.31 km) with 5 parasitoids. In contrast, Silva Macua, which was the farthest site (74.5 km), recorded only 1 parasitoids. This pattern indicates a progressive decline in parasitoid abundance with increasing distance from the release point.The dispersal metrics further confirm this pattern. The mean dispersal distance (MDD) was estimated at 11.105 km, while the maximum dispersal distance (Dmax) reached 74.5 km. The weighted contribution of each site (Di × Ni) shows that Komakoma contributed the highest value (36.55), followed by Silva Macua (74.5), while Mieze contributed 0 due to zero distance.

Together, these findings provide evidence that that *D. longicaudata* was most concentrated near the release site, particularly in Mieze and Komakoma, but also exhibited the ability to disperse over relatively long distances, reaching up to 74.5 km in Silva macua. This suggests successful establishment with a moderate but spatially extended dispersal capacity within the study area.

Table 8. Mean and Maximum Dispersal Distance of parasitoids *Diachasmimorpha longicaudata* across sampling location.

location	Distance Di:(km)	Number of *D. longicaudata* (Ni)	(Di*Ni)
Mieze	0	4	0
Komakoma	7.31	5	36.55
Silva Macua	74.5	1	74.5
Total		10	111.05
MDD	11.105		
Dmax	74.5		

5.0 DISCUSSION

5.1 Effectiveness of M3 alone and M3 in combination with the Male Annihilation Technique in reducing fruit flies density in Boane district

The fruit fly community recorded during the present study was dominated by *B. dorsalis*, which accounted for more than 64% of all captures across treatments, followed by *D. bivittatus*. This finding is consistent with previous studies conducted in Mozambique, particularly in Boane district, where *B. dorsalis* was reported as the most abundant and economically important fruit fly species in mango orchards, largely displacing native Ceratitis species (Canhanga et al., 2020; José et al., 2019). Previous studies in Mozambique and East Africa have identified *D. bivittatus* as an important pest of cucurbit and wild host plants, often occurring alongside *B. dorsalis* in mixed fruit production systems (Mwatawala et al., 2009; Ekesi et al., 2016).

Fruit fly density varied significantly among treatments, clearly demonstrating the influence of management strategies under field conditions in Boane district. The significant reduction in fruit fly density observed under the M3 bait station treatments, particularly when combined with the Male Annihilation Technique (MAT), demonstrates the effectiveness of these management tools in suppressing fruit fly populations. Although the combined M3+MAT treatment did not differ statistically from M3 alone, it consistently recorded the lowest trap catches and fruit fly densities for both *B. dorsalis* and *D. bivittatus*. Similar trends have been reported in Boane, where integrated management approaches combining attract-and-kill technologies, orchard sanitation, and male-targeted control methods resulted in substantial reductions in fruit fly populations and fruit infestation levels (Canhanga et al., 2023).

The effectiveness of MAT against *B. dorsalis* is well documented because methyl eugenol is highly attractive to males, reducing mating opportunities and consequently suppressing population growth (Vargas et al., 2010; Ekesi et al., 2014). Likewise, protein-based bait stations such as M3 target both male and female flies seeking food resources, thereby reducing the reproductive potential of the population. The lower variability observed in treated plots further suggests that these control measures contributed to a more stable suppression of fruit fly populations throughout the sampling period.

In Mozambique, Canhanga et al. (2020) estimated an economic injury level (EIL) of approximately 33.14 flies/trap/week (equivalent to about 4.7 flies/trap/day) in commercial

mango orchards in Manica Province, indicating that populations above this level are likely to cause economically significant losses. Based on those estimates, the treatments were able to reduce the densities below the economic threshold, meaning a successful fruit fly suppression of *B. dorsalis*. The fruit fly density values recorded in the untreated control plots indicate that fruit fly populations remained sufficiently high to pose a significant risk to fruit production in the absence of management interventions.

A similar pattern was observed for *D. bivittatus*, whose FTD decreased significantly from 4.40 flies/trap/day in the control treatment to 1.80 flies/trap/day under M3 bait stations and 0.63 flies/trap/day under the combined M3+MAT treatment. These results indicate that the treatments were effective not only against the dominant species, *B. dorsalis*, but also against *D. bivittatus*, the second most abundant species recorded during the study. The reduction of *D. bivittatus* populations under M3-based treatments may be attributed to the attractiveness of food-based bait stations to both male and female adults seeking protein sources, thereby reducing survival and reproductive potential. Although methyl eugenol-based MAT is species-specific and primarily targets male *B. dorsalis*, the significant reduction in *D. bivittatus* densities observed under the combined treatment suggests that M3 bait stations played the principal role in suppressing this species. Protein-based attractants are known to attract both male and female tephritid flies seeking nutritional resources, resulting in reduced survival and reproductive potential. Therefore, the lower abundance of *D. bivittatus* under the M3+MAT treatment most likely reflects the broad-spectrum activity of the bait stations rather than a direct effect of MAT on this species.

Ecologically, the effectiveness of M3 depends on factors such as host distribution and habitat complexity, which may allow some flies to remain in untreated microhabitats (Ekesi et al., 2006). In addition, environmental conditions such as temperature and time of day influence fly responsiveness to lures, as noted by Tania et al. (2024). From an operational standpoint, lure-based methods often have a limited attraction radius, meaning only a portion of the population is affected while others escape control (Montoya et al., 2007). These combined factors explain why M3 alone reduced fruit fly densities but did not achieve the highest level of suppression. In this case, since both treatments had almost the same density of M3, the increase of 24 MAT devices may have provided only an incremental benefit that was insufficient to produce a statistically significant difference in trap catches.

The observed fruit flies density is consistent with Ekesi et al. (2014), who observed that during bait application, fruit fly catches in orchards ranged between 0.82 and 4.13 FTD in treated plots, representing moderate baseline infestation levels under field condition indicating that lure-based intervention had a measurable impact on population suppression. Similar trends were reported by Vayssières et al. (2015), who demonstrated that protein bait-based strategies significantly reduce fruit fly populations in mango agroecosystems, with treated orchards generally recording moderate infestation levels within a range of approximately 2–5 FTD depending on pest pressure and environmental conditions.

5.2 Infestation levels of fruit flies and parasitism rates in Maputo and Cabo delgado

The infestation indices and parasitism rates observed in this study varied considerably among host fruit species and locations. Guava and tropical almond recorded the highest infestation levels, while citrus and June plum consistently exhibited the lowest infestation indices. Mango showed intermediate values across most infestation parameters. These findings confirm that host fruit suitability strongly influences fruit fly oviposition, larval development, and adult emergence, as previously reported for *B. dorsalis* and other tephritid species (Ekesi & Billah, 2007; Vargas et al., 2015).

The high infestation levels recorded in guava are consistent with its status as a preferred host of *B. dorsalis*. The large numbers of pupae and emerged adults obtained from guava fruits indicate favorable conditions for larval survival and development, likely due to its soft pulp, high moisture content, and nutritional quality (De Meyer et al., 2012; Ekesi et al., 2016; Mwatawala et al., 2009). Tropical almond also supported relatively high infestation levels, although lower than those typically reported for primary hosts such as guava, confirming its role as an important secondary host (Mwatawala et al., 2009; Ekesi et al., 2014, 2016). Mango, another well-known host of fruit flies, showed moderate infestation levels, reflecting the influence of cultivar susceptibility, fruit availability, and local ecological conditions on infestation intensity (Vayssières et al., 2008; Ekesi et al., 2011, 2016).

In contrast, citrus and June plum exhibited significantly lower infestation levels. Citrus fruits possess physical and chemical defenses, including thick rinds and volatile compounds such as limonene, which reduce oviposition success and larval survival (Rattanapun, 2009; Vargas et al., 2015). Similarly, the firmer fruit structure and lower suitability of June plum may limit fruit fly development, resulting in reduced pupal production and adult emergence (Mwatawala et al., 2006, 2009).

The variation in parasitism rates among host fruits and locations further demonstrates the influence of host characteristics on biological control. In Maputo province, parasitism was exclusively attributed to the native parasitoid *Psyttalia cosyrae*, with moderate parasitism levels recorded across host fruits. The highest parasitism rates were observed in Picoco site in citrus (13.33%) and in Umbeluzi site in guava (11.88%), suggesting that fruit structure may facilitate host location and access by parasitoids. Similar observations have been reported in African fruit production systems, where parasitism success is strongly influenced by fruit characteristics and host density (Ekesi et al., 2009; Mohammed et al., 2012).

The parasitism rates recorded in Cabo Delgado province showed clear variation among sampling sites, despite all observations being based on a single host fruit (mango). The native parasitoid *Psyttalia cosyrae* was present across all sites but exhibited generally low parasitism rates, ranging from 3.16% in Komakoma to 10.93% in Metuge. Intermediate values were observed in Silva Macua (9.09%), Chiure (5.34%), Metoro (4.08%), and Mieze (5%). Similar low to moderate parasitism levels of *P. cosyrae* have been reported in previous studies across Africa, where field parasitism rarely exceeds 15% due to environmental constraints and limited host accessibility (Mohamed et al., 2016; Ekesi et al., 2011). The variation observed among sites in this study may therefore be attributed to differences in microclimatic conditions, host density, and orchard management practices, which are known to influence parasitoid efficiency. Higher parasitism in Metuge and Silva Macua suggests relatively favorable ecological conditions, while lower values in Komakoma and Metoro may reflect suboptimal environments for parasitoid activity.

In addition to the native species, the exotic parasitoid *Diachasmimorpha longicaudata* was recorded in a limited number of sites, showing a spatially restricted but important contribution to parasitism. It was present in Mieze (6.55%), Komakoma (5.15%), and Silva Macua (4.76%), where it achieved parasitism rates comparable to or higher than those of the native parasitoid at the same sites. This finding is consistent with previous studies demonstrating that *D. longicaudata* often exhibits higher parasitism efficiency than indigenous parasitoids due to its strong host-searching ability and adaptability (Ekesi et al., 2011; Mwatawala et al., 2009). In several African countries, including Kenya and Tanzania, parasitism rates of *D. longicaudata* have been reported to reach 20–60% under favorable conditions, significantly contributing to fruit fly suppression. Although the parasitism rates recorded in this study are relatively lower, likely due to early establishment and limited

dispersal, the presence of *D. longicaudata* in multiple sites indicates its successful establishment and potential for expansion.

Taken together, these findings suggest that parasitism dynamics in Cabo Delgado are shaped by both spatial variability and parasitoid species composition. The highest parasitism rate recorded in the study was in Metuge (10.93%) by *P. cosyrae*, while the lowest was observed in Komakoma (3.16%). The comparatively higher performance of *D. longicaudata* at certain sites supports previous evidence of its effectiveness as a biological control agent, although its current distribution remains limited. Over time, its dispersal and establishment may enhance overall parasitism levels and improve the biological control of fruit fly populations in the region

Comparatively, Maputo province exhibited higher and more stable parasitism rates for the native parasitoid across multiple fruit types, particularly in citrus and guava, whereas Cabo Delgado showed generally lower parasitism by the native species but introduced the exotic parasitoid, which displayed comparatively higher performance in specific sites such as Komakoma. Overall, the highest parasitism rate recorded in the study was in citrus (13.33% in Maputo), while the lowest was observed in Komakoma for *P. cosyrae* (3.16%). These findings indicate that parasitism dynamics are strongly influenced by location, host fruit type, and parasitoid species, with native parasitoids performing better in diversified fruit systems of Maputo and the exotic parasitoid showing localized success in mango systems of Cabo Delgado.

The relationship between infestation and parasitism patterns suggests that host fruit characteristics influence not only fruit fly development but also parasitoid performance. Fruits with softer tissues and favorable volatile profiles may support high fruit fly infestations while simultaneously facilitating parasitoid host location. Conversely, fruit traits such as thick peels, dense pulp, or unsuitable internal structures can limit both infestation and parasitism success (Mohamed et al., 2006; Mohammed et al., 2019). Furthermore, native parasitoids such as *P. cosyrae* appear less effective against *B. dorsalis*, partly due to host immune defenses and mismatches between parasitoid oviposition and host developmental stages (Mohamed et al., 2003, 2006, 2016).

Interestingly, F*opius arisanus* was not detected during the study, despite previous reports of its establishment following releases in Mozambique. Its absence may reflect low population

densities, failure to persist over time, environmental constraints, competition with other parasitoids such as *D. longicaudata*, or limited availability of suitable host stages during sampling (Cugala et al., 2016; Harbi et al., 2018). Because *F. arisanus* is an egg-larval parasitoid, its success depends on close synchronization with host egg availability, which may restrict its persistence under field conditions.

Overall, the findings indicate that parasitism rates varied across sampling locations in Cabo Delgado province, reflecting differences in parasitoid activity and effectiveness. While *P. cosyrae* was the most widely distributed species across all sites, *D. longicaudata* showed higher parasitism rates than *P. cosyrae* in specific locations such as Mieze and Komakoma, indicating its localized potential in contributing to fruit fly suppression. In general, parasitism levels remained low to moderate despite high host availability in some areas, suggesting that parasitoid performance may be influenced by local environmental conditions. These results highlight the importance of strengthening biological control strategies alongside improved orchard management practices for sustainable fruit fly control.

5.3 Dispersal distance of *D. longicaudata* in Cabo delgado Province

The dispersal patterns of *D. longicaudata* released during this study indicate that their distribution is strongly influenced by distance from the release point. The highest number of *D. longicaudata* was recorded close to the release sites Mieze and Komakoma, while fewer individuals were detected at farther distances (Silva Macua). The mean dispersal distance (MDD) was calculated at 11.105 km, with a maximum dispersal distance (Dmax) of 74.5 km.

These findings showed that the majority of *D. longicaudata* individuals were determined near the release points, with their abundance decreasing progressively with increasing distance. This suggests that dispersal was spatially limited and strongly influenced by proximity to the release sites. These results are consistent with findings by Sivinsk et al. (2000), who reported that parasitoids tend to remain close to release points due to host availability and energy conservation during foraging. Similarly, Montoya et al. (2007) found that *D. longicaudata* reveals localized dispersal behavior, preferring to exploit nearby host patches before expanding to distant areas. In addition, Vargas et al. (2001) demonstrated that environmental factors such as host density, temperature, and habitat structure significantly influence parasitoid movement, often resulting in higher densities near release zones. This behavior explains why sites such as Mieze (0 km) and Komakoma (7.31 km) recorded higher parasitoid numbers, while only a few individuals reached distant locations like Silva Macua

(74.5 km), despite the relatively high maximum dispersal distance (Dmax). Therefore, the moderate mean dispersal distance (MDD = 11.105 km) observed in this study reflects a typical dispersal pattern where most individuals remain close to the origin while only a small proportion disperses over long distances.

The dispersal pattern observed in this study is consistent with findings from other regions, although some differences can be explained by ecological conditions. For instance, studies in Kenya (Ekesi et al., 2006; Mohammed et al., 2019) reported that *D. longicaudata* tends to remain concentrated near release points, similar to the present findings, due to high host availability and favorable environmental conditions. In contrast, research conducted in Hawaii (Vargas et al., 2001) showed a wider dispersal range, likely due to more stable agro-ecosystems and continuous fruit production throughout the year. These comparisons suggest that while *D. longicaudata* generally exhibits limited dispersal, its spread can vary depending on habitat structure and host distribution.

Overall, these comparisons highlight that parasitoid dispersal and establishment are strongly influenced by local ecological conditions found in Cabo Delgado especially in Pemba district.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- This study demonstrated that fruit fly populations were significantly lower in treated areas (M3 alone and M3 + MAT) compared to untreated areas. Among the treatments, the combination of M3 and the Male Annihilation Technique (MAT) proved to be the most effective, confirming that using integrated control methods is more effective than using a single method.
- Fruit infestation levels vary depending on the type of fruit, with guava and tropical almond having the highest infestation, citrus and june plum showing very low infestation, and mango having moderate levels. The native parasitoid *P. cosyrae* were present and had low parasitism rates, while introduced parasitoid *D. longicaudata* showed higher parasitism rates in some cases, especially in mango, indicating better potential for biological control.
- *D. longicaudata* numbers were higher near the release sites and decreased with increasing distance. This indicates that their movement is limited, and their effect is mainly concentrated around the areas where they were released.

6.2 Recommendations

- Farmers are encouraged to adopt basic orchard sanitation practices, including regular collection and proper disposal of fallen and infested fruits, as well as timely harvesting, to reduce fruit fly populations and interrupt their life cycle.
- In addition, farmers and agricultural stakeholders should integrate M3 bait stations with the Male Annihilation Technique (MAT) to improve fruit fly control efficiency, reduce infestation levels, and minimize crop losses.
- Regular monitoring of fruit fly populations and fruit infestation should be encouraged, so that farmers can take action early and apply control measures at the right time.
- Biological control programs should be strengthened by increasing the release and protection of parasitoids such as *Fopius arisanus* and *Diachasmimorpha longicaudata* to improve their effectiveness.
- Parasitoid releases should be expanded to more locations to improve their distribution and increase their impact across wider areas in Mozambique.

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8.0 APPENDICES

Appendix 1: Analysis of Variance (ANOVA) and Tukey Mean Separation for Fruit fly Trap Density (FTD).

a) Fruit fly Trap Density for *Bactrocera dorsalis* analysis of variance

```
> print(anova_results)
            Df Sum Sq Mean Sq F value  Pr(>F)
Treatment    2    647   323.5   17.02 1.1e-06 ***
Residuals   66   1255    19.0
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

b) Tukey mean separation for Fruit fly Trap Density for *Bactrocera dorsalis*

```
> print(summary_data)
  Treatment mean_FTD        SE      FTD groups ANOVA_p
1   Control 8.683230 1.2609113 8.683230      a       0
2        M3 4.068323 0.9018489 4.068323      b       0
3    MAT+M3 1.254658 0.2771088 1.254658      b       0
```

c) Fruit fly Trap Density for *Dacus bivittatus* analysis of variance

```
> print(anova_results)
            Df Sum Sq Mean Sq F value  Pr(>F)
Treatment    2  171.4   85.68   7.261 0.00141 **
Residuals   66  778.8   11.80
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
>
```

d) Tukey mean separation for Fruit fly Trap Density for *Dacus bivittatus*

```
> print(summary_data)
  Treatment  mean_FTD        SE       FTD groups ANOVA_p
1   Control 4.3975155 1.0353109 4.3975155      a  0.0014
2        M3 1.7950311 0.6531627 1.7950311      b  0.0014
3    MAT+M3 0.6273292 0.2013785 0.6273292      b  0.0014
>
```

Appendix 2: Infestation Indices and Non-Parametric Statistical Analysis of *Bactrocera dorsalis* across Different Host Fruits

a) Mean (± SE) of Pupae and Adult Infestation Indices per Fruit and per kg across Different Fruit Types.

```
> print(summary_stats)
# A tibble: 5 × 9
  fruit_type     mean_pupae se_pupae mean_pupae_index se_pupae_index mean_adults
  <chr>               <dbl>    <dbl>            <dbl>          <dbl>       <dbl>
1 Citrus              0.4      0.101             3.20          0.854       0.303
2 Guava              12.0      0.764           198.           11.3         9.22
3 Juneplum            0.554    0.163            11.2           3.34        0.223
4 Mango               2.29     0.656            23.6           6.48        1.30
5 Tropical Almo…      9.63     1.00            353.           35.0         7.73
# ℹ 3 more variables: se_adults <dbl>, mean_adult_index <dbl>,
#   se_adult_index <dbl>
> print(summary_stats, width = Inf)
# A tibble: 5 × 9
  fruit_type      mean_pupae se_pupae mean_pupae_index se_pupae_index
  <chr>                <dbl>    <dbl>            <dbl>          <dbl>
1 Citrus               0.4      0.101             3.20          0.854
2 Guava               12.0      0.764           198.           11.3
3 Juneplum             0.554    0.163            11.2           3.34
4 Mango                2.29     0.656            23.6           6.48
5 Tropical Almond      9.63     1.00            353.           35.0
  mean_adults se_adults mean_adult_index se_adult_index
        <dbl>     <dbl>            <dbl>          <dbl>
1       0.303    0.0827             2.44          0.697
2       9.22     0.645            152.            9.50
3       0.223    0.101              4.63          2.16
4       1.30     0.359             13.5           3.50
5       7.73     0.877            283.           30.9
```

b) Kruskal–Wallis tests for infestations

```
> ####KRUSKAL WALLIS TESTS
> kruskal.test(`pupae infestation` ~ fruit_type, data=data)

        Kruskal-Wallis rank sum test

data:  pupae infestation by fruit_type
Kruskal-Wallis chi-squared = 203.66, df = 4, p-value < 2.2e-16

>
> kruskal.test(`Pupae Infestation Index per Kilogram (Pupae/kg)` ~ fruit_type, data=data)

        Kruskal-Wallis rank sum test

data:  Pupae Infestation Index per Kilogram (Pupae/kg) by fruit_type
Kruskal-Wallis chi-squared = 226.17, df = 4, p-value < 2.2e-16

>
> kruskal.test(`adults per fruit` ~ fruit_type, data=data)

        Kruskal-Wallis rank sum test

data:  adults per fruit by fruit_type
Kruskal-Wallis chi-squared = 204.99, df = 4, p-value < 2.2e-16

>
> kruskal.test(`Adult Infestation Index per Kilogram (Adults/kg)` ~ fruit_type, data=data)

        Kruskal-Wallis rank sum test

data:  Adult Infestation Index per Kilogram (Adults/kg) by fruit_type
Kruskal-Wallis chi-squared = 227.37, df = 4, p-value < 2.2e-16
```

C) Dunn's test for infestations

i) Pupae Infestation Index per fruits (Pupae per fruits)

```
> letters_pupae <- generate_letters("pupae infestation")
  Kruskal-Wallis rank sum test

data: x and g
Kruskal-Wallis chi-squared = 203.6611, df = 4, p-value = 0


                    Dunn's Pairwise Comparison of x by g
                                (Bonferroni)
Col Mean-
Row Mean |      Citrus       Guava    Juneplum       Mango
---------+----------------------------------------------------
   Guava |  -13.03959
         |    0.0000*
         |
Juneplum |  -1.634103    3.925996
         |     1.0000      0.0009*
         |
   Mango |  -2.637892    7.789345    0.216669
         |     0.0834      0.0000*      1.0000
         |
Tropical |  -8.773686    0.615892   -3.294061   -5.628253
         |    0.0000*      1.0000      0.0099*     0.0000*

α = 0.05
Reject Ho if p ≤ α, where p = Pr(|Z| ≥ |z|)
```

ii) Pupae Infestation Index per Kilogram (Pupae per kg)

```
> letters_pupae_index <- generate_letters("Pupae Infestation Index per Kilogram (Pupae/kg)'
  Kruskal-Wallis rank sum test

data: x and g
Kruskal-Wallis chi-squared = 226.1745, df = 4, p-value = 0
```

Dunn's Pairwise Comparison of x by g
(Bonferroni)

Col Mean-Row Mean	Citrus	Guava	Juneplum	Mango
Guava	-12.54057 0.0000*			
Juneplum	-2.000606 0.4543	3.346713 0.0082*		
Mango	-2.280756 0.2256	7.747440 0.0000*	0.747083 1.0000	
Tropical	-11.11802 0.0000*	-2.087772 0.3682	-4.239104 0.0002*	-7.973332 0.0000*

```
α = 0.05
Reject Ho if p ≤ α, where p = Pr(|Z| ≥ |z|)
```

iii) Adults Infestation Index per fruits (Adults per fruit)

```
> letters_adults <- generate_letters("adults per fruit")
  Kruskal-Wallis rank sum test

data: x and g
Kruskal-Wallis chi-squared = 204.988, df = 4, p-value = 0
```

Dunn's Pairwise Comparison of x by g
(Bonferroni)

Col Mean-Row Mean	Citrus	Guava	Juneplum	Mango
Guava	-12.92275 0.0000*			
Juneplum	-1.102202 1.0000	4.408077 0.0001*		
Mango	-2.340011 0.1928	7.993793 0.0000*	-0.138626 1.0000	
Tropical	-8.801670 0.0000*	0.503773 1.0000	-3.801377 0.0014*	-5.888823 0.0000*

```
α = 0.05
Reject Ho if p ≤ α, where p = Pr(|Z| ≥ |z|)
```

iv) Adult Infestation Index per Kilogram (Pupae per kg)

```
> letters_adult_index <- generate_letters("Adult Infestation Index per Kilogram (Adults/kg)")
  Kruskal-Wallis rank sum test

data: x and g
Kruskal-Wallis chi-squared = 227.3665, df = 4, p-value = 0


                    Dunn's Pairwise Comparison of x by g
                                (Bonferroni)
Col Mean-
Row Mean |      Citrus      Guava    Juneplum      Mango
---------+--------------------------------------------
   Guava |  -12.52357
         |     0.0000*
         |
Juneplum |  -1.513440   3.826630
         |     1.0000     0.0013*
         |
   Mango |  -2.034682   7.979917   0.408082
         |     0.4188     0.0000*    1.0000
         |
Tropical |  -11.00421  -1.986207  -4.627380  -8.068140
         |     0.0000*    0.4701     0.0000*    0.0000*

α = 0.05
Reject Ho if p ≤ α, where p = Pr(|Z| ≥ |z|)
```