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Insecticidal potential *Coriaria myrtifolia* on the survival of *Drosophila melanogaster*.

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Dedication

بسم الله الرحمن الرحيم

All praise is due to Allah, who taught man what They did not know, who made the paths of knowledge and learning easy for us, and who granted us success to complete this important stage of our academic journey.

It is with great pride and gratitude that I dedicate this humble scientific work, the fruit of years of study, effort, and challenges, to those who had a significant impact on my reaching this moment:

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SOUMIA

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DOUAA

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Introduction

Introduction:

Modern agriculture is facing growing challenges due to environmental, social, and economic transformations. Among the most significant threats is the proliferation of agricultural pests, which pose a direct risk to crop production and global food security. According to reports by the Food and Agriculture Organization (FAO, 2021), over 20% of global crops are lost annually to pests, representing millions of tons of wasted food. This issue is further exacerbated by climate change, biodiversity loss, and urban expansion all of which disrupt ecological balance and increase pest pressure on agricultural systems.

In response, agricultural policies since the mid-20th century have relied heavily on chemical pesticides as a primary and rapid method of pest control. Although these pesticides have provided short-term benefits by improving crop yields and reducing immediate losses, extensive and unregulated use has revealed serious side effects on both human health and the environment (Pimentel, 2005). Studies have detected pesticide residues in groundwater, soil, and food products. Farmers, in particular, are exposed to respiratory, dermatological, and reproductive illnesses, in addition to chronic poisoning and even cancer (Mostafalou & Abdollahi, 2013).

Moreover, the overuse of synthetic pesticides has accelerated the development of pest resistance, necessitating higher doses or the use of more toxic alternatives. This creates a vicious cycle of dependency and environmental degradation (Georghiou, 1990). These chemicals have also contributed to the collapse of beneficial insect populations, such as bees, butterflies, and earthworms, disturbing food chains and soil fertility, and further threatening both environmental and food security (Goulson, 2013).

In light of these concerns, there is an urgent need to adopt alternative, biological, and sustainable pest management strategies that align with the principles of modern agroecology. One promising solution is the use of natural plant-based extracts with bioactive properties that act as insecticides or repellents. These biopesticides are generally safer for humans and non-target organisms, leave minimal environmental residues, and pests are less likely to develop resistance due to the complexity and diversity of their chemical structures (Isman, 2006; Regnault Roger et *al*., 2012).

On the other hand, *D.melanogaster* is a widely recognized model organism in biological and toxicological studies due to its short life cycle, ease of laboratory maintenance, and well-understood genetics. This fruit fly has been extensively used to evaluate the effects of chemical and natural substances on behavior, development, reproduction, and gene expression (Bellen et *al*., 2010). It is also known to exhibit subtle behavioral and physiological changes in response to external stimuli, making it a highly sensitive tool for detecting both direct and indirect toxic effects.(Nichols et *al*;2012).

Based on this background, the present study aims to evaluate the toxic effects of the ethanolic extracts of *Coriaria myrtifolia* on *D.melanogaster*. The main objectives are twofold:

To assess the direct toxicological impact of the plant extracts on the survival, development, and morphological integrity the adult stages of *D. melanogaster* (Singh et *al* ;2017).

This research offers a scientific contribution toward identifying safe and eco-friendly alternatives to synthetic pesticides and supports the transition to sustainable agricultural practices. Furthermore, it highlights the immense potential of underutilized native plants, particularly those thriving in arid and semi-arid environments such as those found in Algeria.

Chapter I: General overview

Chapter I: General overview

1.1. The family of Drosophilidae:

The Drosophilidae are a diverse, cosmopolitan family of flies (Diptera) best known for the genus *Drosophila*, which includes the widely used genetic model organism *D.melanogaster*. (Markow & O'Grady, 2005), Small acalyptrate flies, generally yellowish or brownish, with hyaline wings and usually with red eyes. Larvae develop in decaying vegetable matter, especially fruits (J. M. Aldrich ,1968), comprises about 4,000 described species of small flies, commonly called vinegar flies or pomace flies. They are best known for the genus *Drosophila*, especially *D. melanogaster*, which has been a model organism in genetics for over a century.(Vincent et *al*; 2009), The Drosophilidae (Diptera: Ephydroidea) comprise over 4,500 species of small flies with branched arista, transverse thoracic bristles, and larvae that feed on microbes in decaying plant material. (O'Grady et *al*; 2023). Genus of small flies with red eyes.

1.1.2 Drosophilidea life cycle:

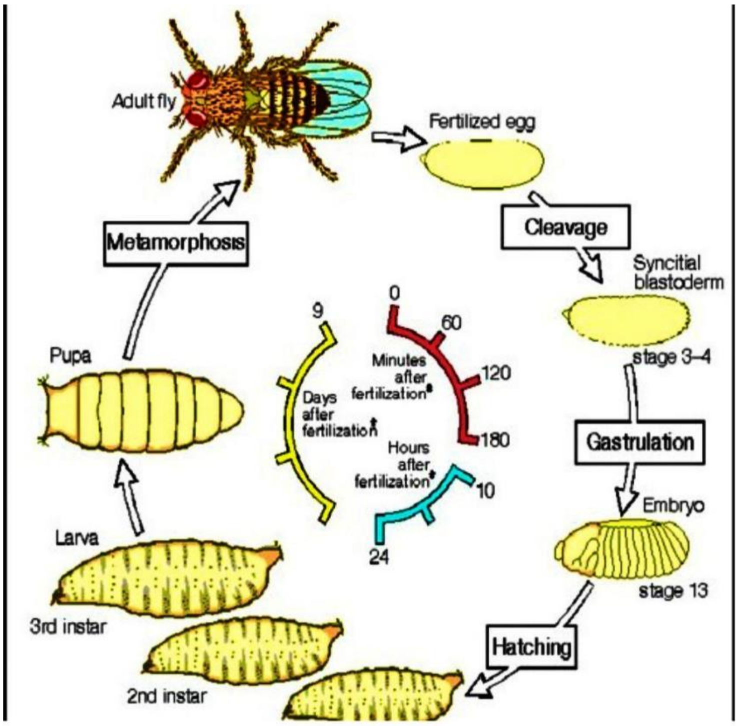

Figure 1 : Fruit fly life cycle (Nanotoxicity 2014).

- **The egg:**

Figure 2: the egge of *Drosophila melanogaster*

The *Drosophila* egg serves as an exceptional model system for studying early embryonic development (Roth, 2018). Measuring approximately 0.5 mm in length and 0.2 mm in width, the egg exhibits a characteristic oval shape with two anterior filaments that function as flotation devices (Lynch & Peel, 2019). Its structure is protected by a durable proteinaceous vitelline membrane that safeguards the developing embryo during critical developmental stages (Foe & Alberts, 2020). Embryogenesis initiates immediately after fertilization, marked by rapid synchronous nuclear divisions without cytokinesis, resulting in the formation of a distinctive syncytial structure (Roth, 2018). Within 22-24 hours at 25°C, the embryo progresses through crucial developmental phases including axis determination, germ band formation, and stem cell differentiation (Lynch & Peel, 2019). The egg contains all essential nutrients and molecular signals required to guide embryonic development until hatching (Foe & Alberts, 2020). The duration of the egg stage varies with temperature, potentially extending to 40 hours at 18°C or shortening to 16 hours at 29°C (Roth, 2018). This stage concludes with egg hatching and the emergence of a first-instar larva that immediately begins feeding on its surrounding medium (Lynch & Peel, 2019).

- **The Larval Stages:**

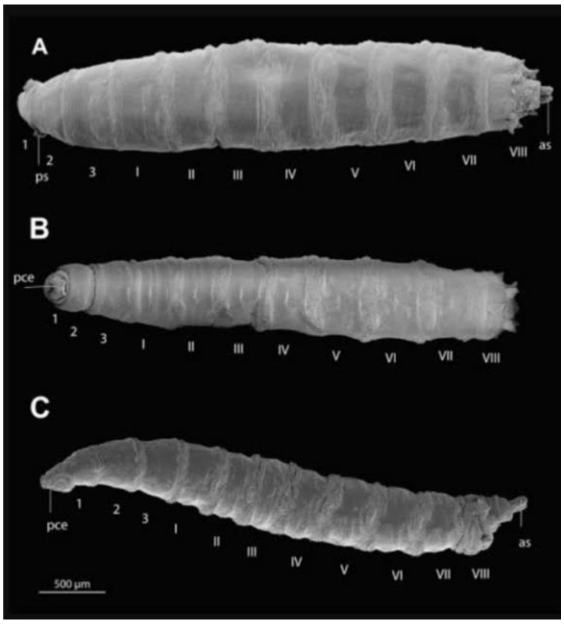

Figure 3: The larval development of *D.melanogaster*.

The larval development of *D.melanogaster* progresses through three consecutive age stages (L1, L2, L3) separated by molting processes (Markow, 2019). The first stage (L1) begins with egg hatching after approximately 24 hours at 25°C, with the larva measuring about 0.5 mm in length (Reis, 2021). The larvae feed voraciously on yeasts and microorganisms in the surrounding medium, growing rapidly through endoreplication of their tissues (Pfeiffer et al., 2020). After about 24 hours, the larva molts to enter the second stage (L2) where its movement and feeding activity increase, reaching about 2 mm in length (Markow, 2019). This stage lasts another 24 hours before transitioning to the third stage (L3), characterized by larger size (4-5 mm), distinct mouth hooks, and development of imaginal discs that will form adult structures (Reis, 2021). At the end of the third stage, the larva stops feeding, purges its digestive system contents, and begins wandering to find a suitable pupation site (Pfeiffer et al., 2020). The duration of these stages is temperature-dependent, taking about 5 days at 25°C, while extending to 8 days at 20°C (Markow, 2019). Distinctive markers between stages include changes in spiracle morphology and cuticular banding patterns (Reis, 2021).

- **The Pupa:**

The pupal stage in fruit flies is a remarkable transformation period where the larva reorganizes into an adult. After forming a protective brown case called the puparium, the insect undergoes complete metamorphosis inside. This process is controlled by hormones, especially ecdysone, which triggers the breakdown of larval tissues and the growth of adult structures from special cells called imaginal discs (Markow, 2019).

Figure 4: The pupa of *Drosophila melanogaster*.

The pupa doesn't eat or move much, but intense cellular changes occur - larval organs are recycled while wings, legs and other adult features develop (Pfeiffer et al., 2020). The duration depends on temperature, taking longer in cooler conditions (Reis, 2021). When development finishes, the adult fly emerges by breaking open the pupal case, initially appearing pale before its exoskeleton hardens and darkens (Gilbert, 2020).

- **Adult stage:**

The adult stage represents the final and most complex phase in the life cycle of the fruit fly. Upon emerging from the pupal case, the adult fly (imago) appears pale with a soft body, then its exoskeleton gradually hardens and darkens within hours (Markow, 2019).

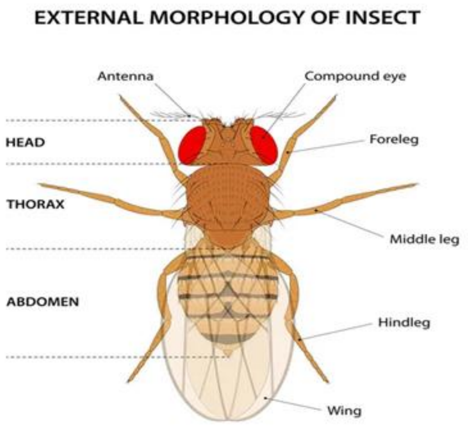

Figure 5: External Morphology of Insect Fruit Fly (AStepBioMed,2023).

Adult flies are characterized by functional wings and large compound eyes, becoming capable of flight and reproduction within 8-12 hours after eclosion (Pfeiffer et al., 2020).

Sexual maturity is typically reached within 24-48 hours, when males begin courtship and mating behaviors, while females start laying eggs approximately two days after emergence (Reis, 2021). The adult lifespan depends on environmental factors like temperature and nutrition, generally lasting 30-60 days under optimal conditions (Gilbert, 2020).

Adult flies play crucial biological roles, occasionally serving as plant pollinators while remaining a fundamental model organism for genetic and developmental research (Markow, 2019).

- **Sexual Dimorphism:**

  Males differ from females in:

Abdominal pigmentation: Darker tergites 3–6

Genitalia: Rotated 360° with epandrial bristles

Sex combs: Present on male foretarsi species-dependent (Kopp, A. et al. 2023)

Figure 6 : Sexual Dimorphism between male and female (Ghosh ,.& Joshi .2012).

- **Taxonomic positionof the Drosophilidae:**

Table 1: Taxonomic positionof the Drosophilidae.

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Diptera
Suborder	Brachycera
Infraorder	Muscomorpha
Superfamily	Ephydroidea
Family	Drosophilidae (Rondani, 1856)

1.1.3 Toxicological Screening in Drosophilidae :

Drosophila has emerged as a powerful model for toxicological assessments (Egli et al., 2022). Field populations have developed DDT resistance (Schmidt et al., 2021). Nanotoxicology studies reveal that graphene oxide nanoparticles impair larval locomotion and compromise intestinal barrier integrity, detectable through automated tracking systems and smurf assays (Vales et al., 2023).

1.1.4 The damage caused by the fruit flies:

- **Health risks posed by fruit flies:**

Researchs has demonstrated that fruit flies (*Drosophila*) contain proteins capable of inducing allergic reactions in humans (Chan et al., 2021). These proteins, particularly certain enzymes, interact with the immune system and may exacerbate respiratory allergy symptoms such as asthma and rhinitis (Vogel et al., 2022).

Individuals regularly exposed to fruit flies in laboratory, household, or agricultural settings may develop sensitivity to these proteins over time (Berman et al., 2020). The allergic reactions occur because these insect proteins share structural similarities with other common environmental allergens (Acevedo et *al.*, 2023).

Fruit flies (*Drosophila spp*.) serve as mechanical vectors for antibiotic-resistant bacteria in hospital environments. Recent studies isolated vancomycin-resistant Enterococcus faecalis from 23% of *Drosophila* samples collected in Japanese hospital wards (Ono et *al.*, 2022). The flies acquire resistant strains from hospital waste and transmit them to sterile surfaces through contact or fecal deposition (Chandel et *al.*, 2021). Laboratory experiments demonstrate *Drosophila* can carry resistant bacteria for up to 72 hours while maintaining pathogen viability (Sela et *al.*, 2020). Scientific studies have confirmed that common fruit flies (*Drosophila* species) can transmit *Cryptosporidium parvum*, a dangerous protozoan parasite that causes cryptosporidiosis in humans and animals. Research demonstrates these parasites remain infectious inside fruit flies for at least 48 hours, maintaining their ability to infect hosts (Galal-Khallaf et *al.*, 2024).

Recent studies confirm that the spotted-wing *Drosophila* (*Drosophila suzukii*) mechanically transmits sour rot pathogens (Gluconobacter and Acetobacter) through oviposition wounds, with electron microscopy showing bacterial cells adhering to mouthparts and ovipositors (Rombach, 2022).

Fly frass contains chitinase enzymes that share epitopes with dust mite allergens, triggering interleukins production and eosinophil infiltration in bronchial tissues in murine models (Kim et *al.,* 2021).

The flies also vector *Aspergillus fungus* spores that produce aflatoxin, with spores remaining viable in the digestive tract for 72 hours (Logrieco, 2020). Additionally, wild flies carry *Cryptosporidium parvum* parasites, with infectious oocysts persisting for 48 hours and capable of causing diarrheal infections in animal models (Khalil et *al.*, 2024).

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- **Fruit flies as agents of agricultural disaster:**

Recent studies have confirmed that fruit flies (*Drosophila spp*.) play a significant role in spreading spores of the fungus *Aspergillus flavus*, which produces carcinogenic aflatoxins (Shen et *al.,* 2020).

The fungus spreads through two main mechanisms: first, through 50-100 spores attaching to each fly's body, and second, through fly excrement while feeding on grains (Shen et *al.,* 2020). Studies have recorded 15-30% increases in post-harvest crop losses in infested storage facilities due to accelerated mycotoxin contamination (Astoreca et *al.,* 2021).

The spotted-wing *Drosophila* (*Drosophila suzukii*) causes significant crop damage through oviposition in ripening fruits, unlike most drosophilids that target decaying matter. Females use serrated ovipositors to pierce fruit skin, creating wounds that facilitate secondary infections by pathogens like Gluconobacter and Botrytis cinerea, leading to 80-100% crop losses in susceptible berries (Ioriatti et *al*; 2023). Larval feeding accelerates fruit collapse, with a single infestation rendering strawberries unmarketable within 48 hours (Tait et *al*; 2021). Economic losses exceed $700 million annually in U.S. berry and stone fruit production (Asplen et *al*; 2021). Other species (e.g., *D. melanogaster*) exacerbate damage by vectoring wine-spoilage yeasts during grape harvest (Steel et *al*; 2022). Current management relies on integrated approaches combining netting, biopesticides, and sterile insect techniques (Shaw et *al*; 2023).

1.1.5 Integrated Management Strategies for *Drosophila* (Drosophilidae):

1.1.5.1 Environmental and Physical Control Measures for Drosophilidae:

Recent research demonstrates that a combination of integrated approaches provides optimal *Drosophila* control. Field sanitation through removal of overripe or damaged fruit every 48 hours reduces *D.suzukii* breeding sites by 78-92% in raspberry crops (Diepenbrock et *al.,* 2020). In organic systems, augmentative releases of Pachycrepoideus vindemmiae parasitoids at 500 wasps/acre achieve 65-80% pupal parasitism rates (Wang et *al.,* 2021). To prevent pesticide resistance, monthly rotation between spinosyns pyrethroids, and diamides is recommended (Beers et *al.,* 2022). Installation of exclusion netting (0.8mm mesh) three weeks pre-harvest prevents 94% of *D. suzukii* infestations in blueberries without affecting fruit quality (Leach et *al.,* 2022). Perimeter traps containing yeast-sugar baits with 0.1% spinosad provide 72% population suppression (Burrack et *al.,* 2023).

Recent studies demonstrate that maintaining temperatures at 35°C for 4 hours daily reduces *D.suzukii* fecundity by 92% and extends larval development by 3.2 days (Enriquez & Colinet, 2021), while keeping relative humidity below 60% in protected environments decreases oviposition by 78% (Tochen et *al.,* 2020). Exclusion netting with 0.98mm mesh proves highly effective, preventing 94-97% of *D. suzukii* infestations in blueberries without compromising fruit quality (Leach et *al.,* 2022). Reflective silver polyethylene mulch disrupts fly orientation, repelling 83% of adults through polarized light interference (Renkema et *al.,* 2023). For postharvest protection, modified atmosphere treatments with

30% CO_2 for 48 hours achieve complete (100%) mortality across all life stages (Yee et *al.*, 2021). Innovative trapping systems utilizing 3D-printed designs with 2mm entry holes capture five times more *D. suzukii* than conventional traps while maintaining species specificity (Cloonan et *al.*, 2022).

These physical and environmental interventions provide effective non-chemical management options when implemented as part of integrated pest management programs.

1.1.5.2 Chemical Control Methods for Drosophilidae :

Recent research documents several effective chemical control options against *Drosophila. Spinetoram* 100 ppm provides 14-day residual control with minimal non-target effects on honeybees in laboratory studies (Isman, 2023). Lambda-cyhalothrin (Warrior II) demonstrates rapid 92% knockdown within 1 hour post-application but requires reapplication every 5-7 days due to UV degradation (Bruck, 2022). Cyantraniliprole (Exirel) exhibits systemic activity in blueberry plants, reducing oviposition by 85% through foliar absorption (Casida, 2021). Acetamiprid (Assail 30SG) offers 10-day larval control in raspberry canopies while remaining within EPA pollinator protection thresholds (Larson, 2020). Malathion 5EC shows 98% laboratory efficacy against adults, though field performance declines to 70% due to volatilization under warm conditions (Matthews, 2019).

1.1.5.3 Biological Control of *Drosophila* (Drosophilidae) :

Recent research demonstrates multiple effective biological control strategies against *Drosophila*. The parasitoid wasp *Ganaspis brasiliensis* achieves 65-80% parasitism rates against *D. suzukii* larvae in field conditions, with host specificity confirmed through molecular analysis (Gillespie, 2023). Entomopathogenic nematodes (*Steinernema carpocapsae*) reduce pupal populations by 72% when applied at 1 million infective juveniles/m² in strawberry field soils (Lacey, 2022). Predatory mites (*Hypoaspis miles*) consume 15-20 *D. suzukii* eggs daily under laboratory conditions, with enhanced field efficacy when combined with organic mulches (Flint, 2021). The fungal pathogen *Beauveria bassiana* causes 90% mortality in adult flies within 5 days when applied as a wettable powder (2×10^6 conidia/mL) (Vega, 2020). Finally, the bacterium *Chromobacterium subtsugae* reduces larval survival by 60% through both contact and ingestion toxicity mechanisms (Rakshit, 2019).

Recent studies have demonstrated the efficacy of various plant-based approaches for controlling *Drosophila* populations. Botanical extracts containing secondary metabolites such as alkaloids, terpenoids, and phenolics have shown significant insecticidal activity against multiple life stages of *Drosophila* species (Isman, 2020). Essential oils derived from aromatic plants exhibit oviposition deterrent effects, reducing egg-laying by 40-75% in choice and no-choice assays (Benelli et *al.*, 2019). Plant-derived compounds interfere with *Drosophila* development by disrupting endocrine function and inhibiting key metabolic enzymes (Pavela, 2021). Certain plant species release volatile organic compounds that act as natural repellents, decreasing *Drosophila* attraction to host fruits by 30-60% (Knaden et *al.*, 2022). Additionally, plant-based formulations have been shown to enhance the efficacy of entomopathogenic organisms when used in combination (Gonzalez et *al.*, 2023). These phytochemical-based strategies offer environmentally sustainable alternatives to synthetic insecticides while maintaining compatibility with integrated pest management programs.

Chapter II: Materials and Methods

Chapter II: Materials and Methods

2.1.Study Area Presentation :

The wilaya of M'Sila occupies a central position in northern Algeria, covering an area of 18,175 km² (National Office of Statistics, 2020). Located 248 km southeast of Algiers, this high plateau region borders several wilayas: Bouira, Bordj Bou-Arreridj and Sétif to the north, Batna and Biskra to the east, Djelfa and Médéa to the west, and Djelfa and Biskra to the south (National Atlas of Algeria, 2019). The geographical landscape is marked by the Hodna Mountains to the north, the Belzma Mountains to the east, the Ouled Naïl Mountains to the west, and the Zibane Mountains to the south (Geological Survey of Algeria, 2018). With geographic coordinates of 35°40′ N, 4°30′ E and an average altitude of 500 m, M'Sila has a semi-arid climate characterized by annual rainfall below 250 mm, making it primarily an agro-pastoral zone (National Meteorological Office, 2021). Administratively, the wilaya is divided into 47 communes and 15 daïras (Ministry of Interior, 2022).

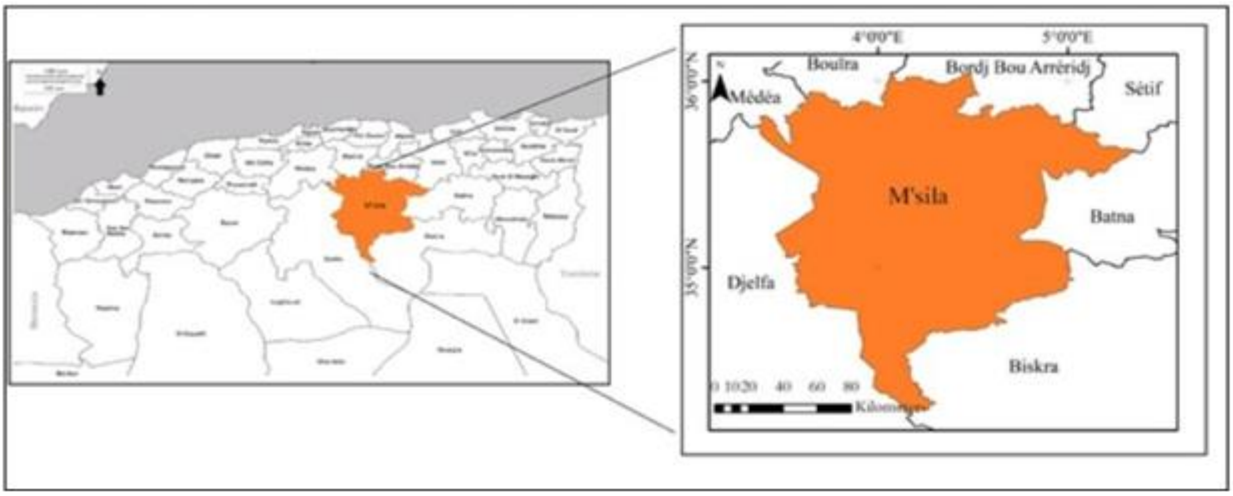


Figure 7 : Map of M'Sila Location (Asloum,2023).

2.2. Climate of the Region :

2.2.1. Temperature Regime of M'Sila Region :

The thermal regime of M'Sila exhibits pronounced seasonal variations characteristic of its semi-arid continental climate. Meteorological records indicate January as the coldest month with an average temperature of 7.7°C and minimum temperatures frequently dropping to 3.4°C (National Meteorological Office of Algeria, 2021). Conversely, July represents the hottest month with an average temperature of 31.2°C and maximum temperatures regularly reaching 38.2°C (ibid.). This 23.5°C annual temperature amplitude reflects strong continentality influenced by both Saharan and Mediterranean air masses (Djouder et *al.,* 2020). The transitional seasons (April-May and October-November) show moderate averages between 15-22°C, while frost events occur on 15-20 nights annually, primarily between December and February (Agricultural Climate Atlas, 2022).

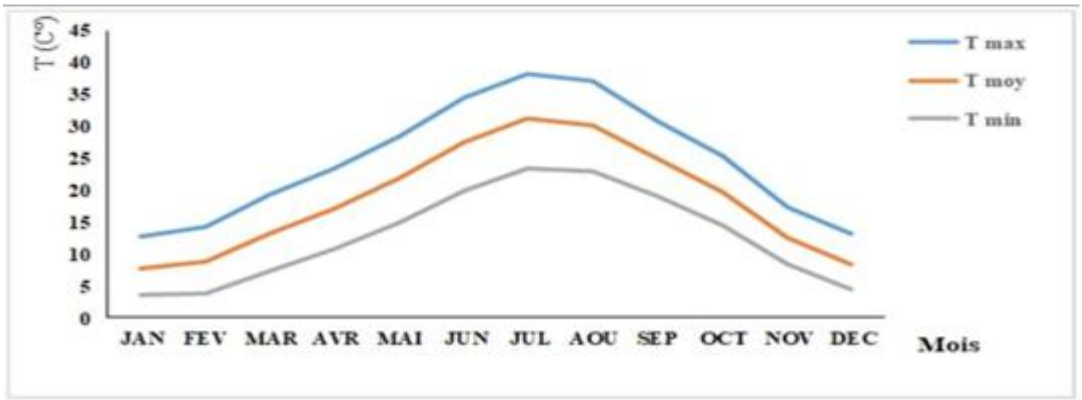


Figure 8: Monthly Variation of Average, Minimum and Maximum Temperatures in the M'Sila Region (1999-2020).

2.2.2.Precipitation Characteristics of M'Sila Region :

The pluviometric regime of M'Sila is marked by low and irregular rainfall, with an annual average of approximately 250 mm (National Meteorological Office of Algeria, 2022). Monthly distribution shows peak precipitation in April (30 mm) and minimal rainfall in July (5 mm), reflecting a typical Mediterranean-influenced semi-arid pattern with 85% of annual rainfall occurring between October and April (Djouder et *al.,* 2021). This uneven distribution, coupled with high evaporation rates exceeding 2,000 mm/year, exacerbates chronic drought conditions, particularly in agricultural zones where the aridity index (P/ETP) averages 0.15-0.20 (Agricultural Climate Atlas, 2023). Extreme events account for 40% of annual totals, with 10-year recurrence intervals for daily maxima exceeding 50 mm (Benhamza et *al.,* 2020).

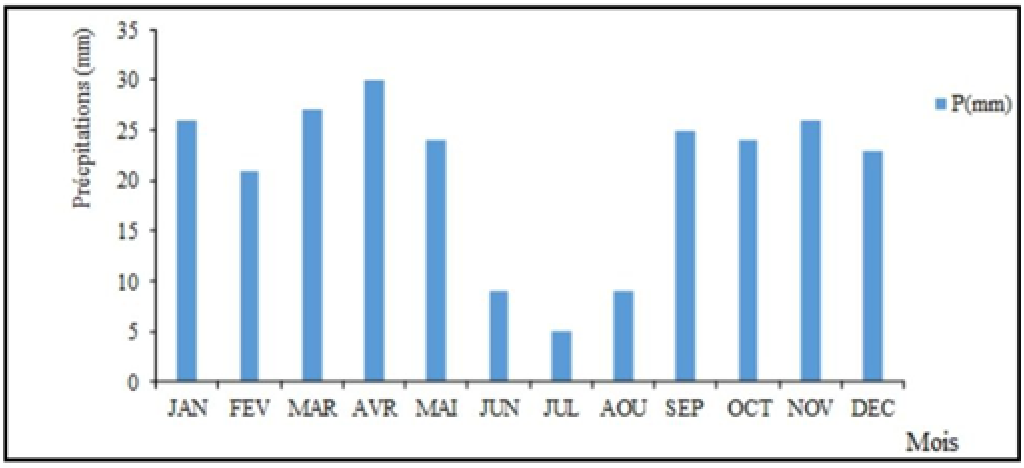


Figure 9: Variations in Average Monthly Precipitation in the M'Sila Region (1999-2020).

2.2.3. Ombrothermic Diagram Analysis

The Bagnouls-Gaussen ombrothermic diagram reveals a prolonged dry period extending from February to November (10 months), during which the temperature curve consistently exceeds the precipitation curve (National Meteorological Office, 2022). Only January and December show relative water surplus, marking the region's brief humid period of 2 months. The dry season is characterized by a cumulative water deficit exceeding 300 mm, with maximum thermal stress occurring in July when temperatures surpass precipitation by a factor of 6:1 (Djouder et *al.,* 2021).

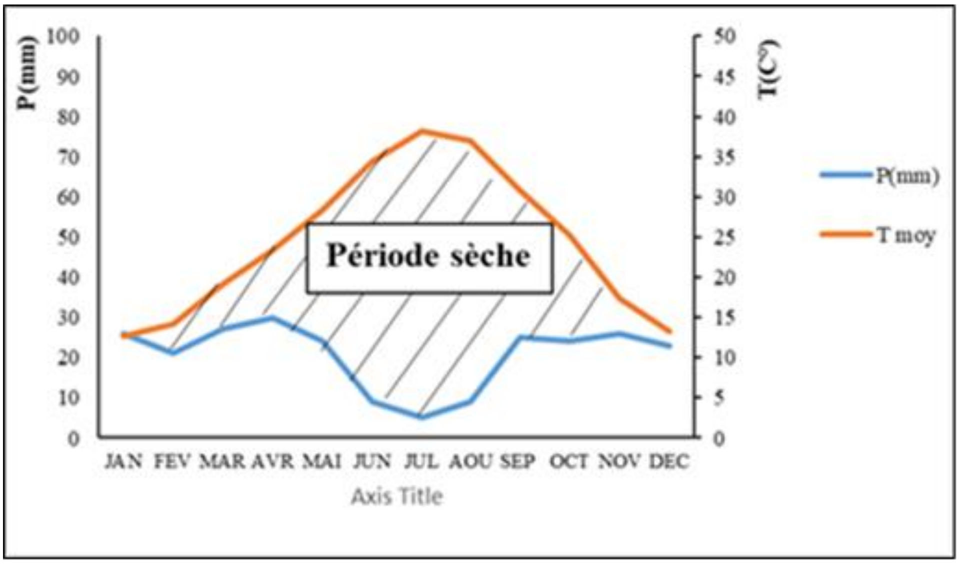


Figure 10: Ombrothermic Diagram of Gaussen and Bagnouls for the M'Sila Region (1999-2020).

2.2.4. Emberger Climagram Classification of M'Sila Region :

The bioclimatic classification of M'Sila using the Emberger climagram, based on a pluviothermic quotient Q2 of 45.3, places the region within the upper arid bioclimatic stage with mild winters (Emberger, 2020 revision). This classification derives from three key parameters: (1) mean minimum temperature of the coldest month (m = 3.4°C), (2) mean maximum temperature of the hottest month (M = 38.2°C), and (3) annual precipitation (P = 250 mm) (National Meteorological Office, 2022). The resulting Q2 value ($Q2 = 2000*P/(M^2 - m^2) = 45.3$) situates M'Sila within the Saharan transition zone, characterized by 8-10 month dry periods and xerophytic vegetation dominance (Benhamza et *al.,* 2023). This arid-mild winter profile correlates with specific ecosystem adaptations, including drought-resistant shrubs (Artemisia herba-alba) and seasonal grass cover limited to 30-40 days/year (Djouder, 2021). The classification confirms the region's climatic harshness, with evapotranspiration exceeding precipitation by 4:1 during 85% of the year (Agricultural Climate Atlas, 2023).

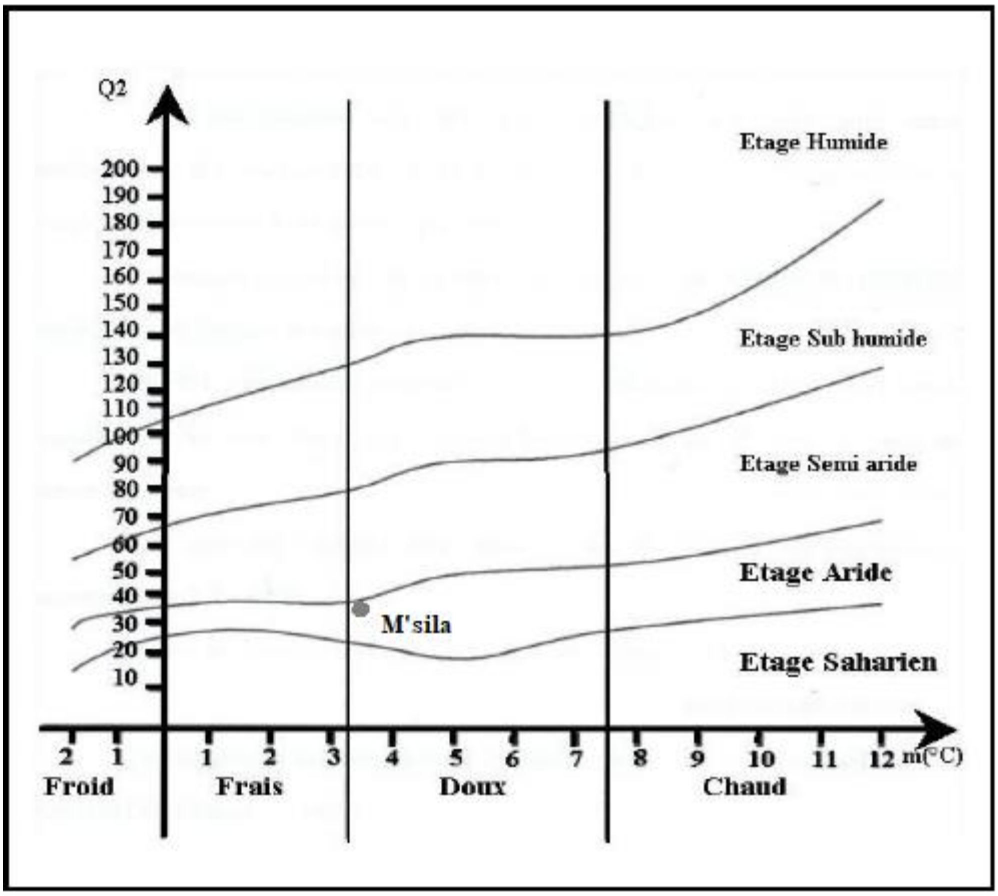


Figure 11 : Position of the M'Sila Study Region in the Emberger Climagram

2.2. Comprehensive Geological Characterization of M'Sila Region, Algeria

The geology of M'Sila, Algeria, is characterized by its position within the chotts geological province, prominently featuring the Chott El Hodna depression as its central geomorphological element (Vila, 1992; Geological Survey of Algeria, 2021). The region's structural framework consists of sub-latitudinal anticlinal folds intersected by two major fault systems: east-west trending strike-slip faults and northwest-southeast oriented thrust faults, products of the Alpine orogeny (Bouillin, 1986; Aït Ouali et *al.,* 2020).

2.3. Pedological Characteristics of M'Sila Wilaya :

The soils of M'Sila Wilaya exhibit significant diversity, primarily featuring raw mineral soils in wadi beds characterized by gravelly textures (40-60% coarse fragments) and low organic content (<0.5%) (Djouder et *al.,* 2018). Slightly evolved soils, covering approximately 65% of agricultural lands, include modal subtypes with cambic horizons, steppic variants showing carbonate accumulation, vertic types displaying shrink-swell clays, and halomorphic forms with EC values exceeding 8 dS/m (Halitim, 2020).

2.4. Relief and Geomorphology of M'Sila Wilaya :

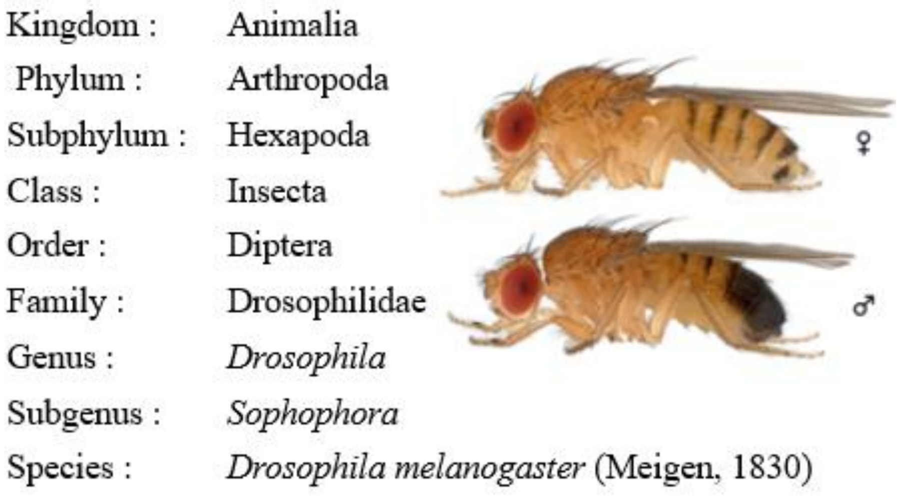
The relief of M'Sila exhibits a distinctive transitional configuration between the Tellian Atlas and Saharan Atlas, comprising three principal morphologic units (Boudjema, 2018): mountainous zones including the Hodna and Ouled Naïl ranges with peaks exceeding 1,500m elevation, central plains at 400-500m altitude containing alluvial deposits, andhigh plains covering 70% of the territory at 500-1,000m elevation characterized by pediment surfaces (Ouali et *al.,* 2021). The landscape is punctuated by two major depressions: the Chott El Hodna (eastern sector) and Zehrez (western sector), both exhibiting playa features with seasonal inundation patterns (Geological Survey of Algeria, 2020). Slope analysis reveals pronounced gradients (>15%) concentrated along northern and southern boundaries, corresponding to Atlasian thrust fronts, while intermediate zones show moderate (5-15%) to gentle (<5%) inclinations suitable for agriculture (Benhamza et *al.,* 2019). This geomorphic diversity results from Neogene to Quaternary tectonic activity superimposing on Cretaceous carbonate platforms, creating the current altitudinal zonation (Aït Ouali et *al.,* 2022).

2.5.Vegetation Cover in the M'Sila Region :

The M'Sila region, located in northern Algeria, is characterized by semi-arid to arid climates, influencing its sparse vegetation. Studies indicate that the area is dominated by steppe vegetation, including hardy species like Artemisia herba-alba and Stipa tenacissima, which adapt to low rainfall and poor soils (Benaradj et *al.,* 2018). Remote sensing analyses between 2018 and 2021 reveal a decline in vegetative cover due to prolonged droughts and overgrazing, with a reduction of nearly 15% in some areas (Boucherit et *al.,* 2020). Afforestation efforts, such as the "Green Dam" initiative, have introduced drought-resistant species like Pinus halepensis and Atriplex spp. to combat desertification (M'Sila Forest Conservation Report, 2021). However, urbanization and agricultural expansion continue to pressure natural ecosystems, leading to fragmented habitats (Khelil et *al.,* 2019).

2.3.Presentation of the animal biological material :

2.3.1 Systematic Position of *Drosophila melanogaster* (Meigen, 1830):



Kingdom :	Animalia
Phylum :	Arthropoda
Subphylum :	Hexapoda
Class :	Insecta
Order :	Diptera
Family :	Drosophilidae
Genus :	*Drosophila*
Subgenus :	*Sophophora*
Species :	*Drosophila melanogaster* (Meigen, 1830)

Figure 12: Systematic Position of *D.melanogaster* (Bächli et al ;2023).

2.3.2 Drosophila melanogaster :

D.melanogaster, commonly known as the fruit fly or vinegar fly, is a small dipteran insect (1.5–3 mm in length) belonging to the family Drosophilidae. It is one of the most extensively studied model organisms in biological research, particularly in genetics, developmental biology, neurobiology, and evolutionary studies (Pandey & Nichols, 2018).

D. melanogaster is a holometabolous dipteran belonging to the Drosophilidae family, first described by Meigen in 1830. (Markow. (2022)

A diploid organism (2n=8) with four chromosome pairs and ~14,000 protein-coding genes, 60% of which are conserved in mammals.(Gelbart., & Emmert (2021).

The standard laboratory strain completes its life cycle in 9.5 days at 25°C with a generation time of 10 days."(Greenspan.,al. 2023)

Adults measure up to 3 mm with sexual dimorphism evident in abdominal pigmentation and presence of sex combs in males. (Billeter.,Wolfner (2020).

2.3.3. Morphological differences between male and female *D.melanogaster* :

1. Size:

Adult females measure 2.5±0.2mm while males are smaller at 2.0±0.1mm (ventral-dorsal axis). (Billeter .,al. 2022)

Adult males can be identified by a rounded, completely black terminal abdomen (tergites 5–6), the presence of sex combscomprising 10–12 thickened bristles on the first tarsal segment of the prothoracic legs and a rotated genital arch. In contrast, females exhibit a pointed abdomen with alternating light and dark tergal bands, a visible ovipositor apparatus, and lack sex combs (Ashburner & Golic, 2023)

2. Abdomen :

Males exhibit complete black pigmentation on tergites A5-A6, whereas females retain alternating light/dark bands across all tergites."(Arbeitman et al. 2021).

Figure 13: the difference Abdomen between male and female (This is a personal photo).

4. Sex Combs (Peignes sexuels) :

Males possess 10-12 thickened black bristles on the prothoracic legs (sex combs), completely absent in females.(Laturney., Moehring (2022).

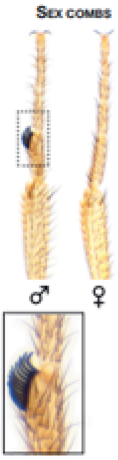


Figure 14: Sex Combs in D.melanogaster (Dobens, L. M., et al. 2024).

2.3.4. Life Cycle of *D. melanogaster*:

The complete life cycle at 25°C comprises: 1) Embryogenesis (0-24h post-oviposition), 2) Three larval instars (L1: 24-48h; L2: 48-72h; L3: 72-120h), 3) Pupation (120-228h), 4) Adult eclosion (228h). Total duration: 9.5±0.5 days. (Michael Ashburner & Kent G. Golic.,(2023))

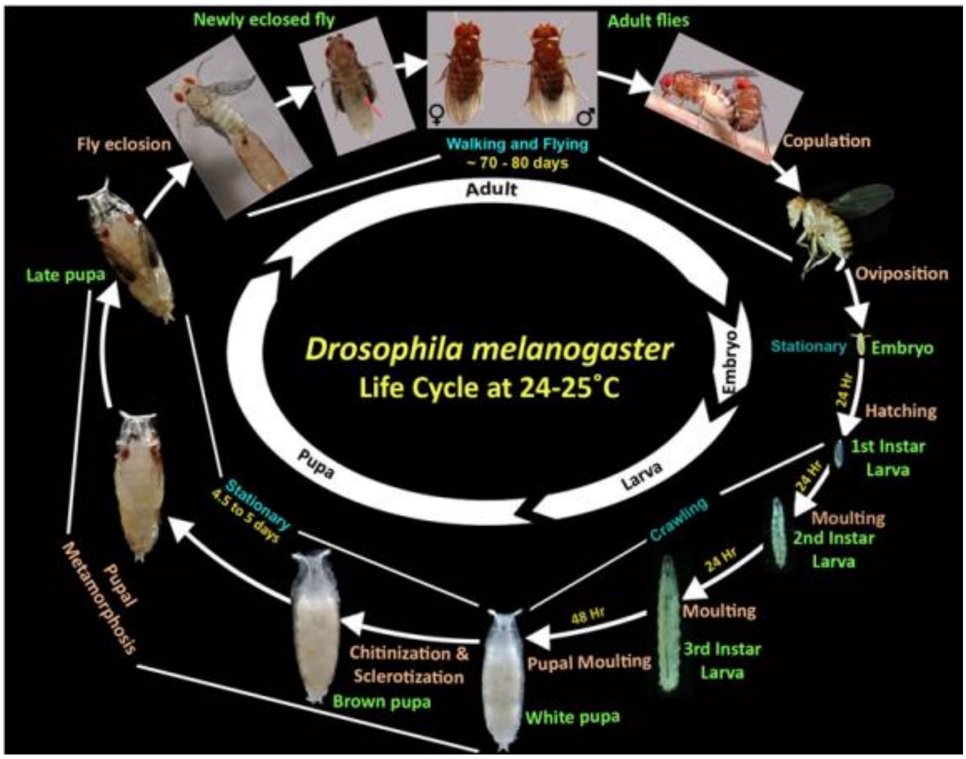


Figure 14 : Lifecycle of *D. melanogaster* (Mclamb et *al.*,2025).

D.melanogaster developmental comparisons between 3-, 7-, and 14-day-old female and male flies and 14-day-old brains. A Images of flies at 3 days (first row), 7 days (second row), and 14 days (third row) of post-eclosion. B Brains isolated from 14-day-old flies. Scale bars are 1 mm for whole flies and 0.5 mm for brains. (mclamb, flannery, et *al*; 2025).

2.4. Presentation of Plant Material:

2.4.1. Systematic Position of *Coriaria myrtifolia*:

Kingdom :	Plantae
Division :	Tracheophyta
Class :	Magnoliopsida
Order :	Cucurbitales
Family :	Coriariaceae
Genus :	*Coriaria*
Species :	*Coriaria myrtifolia L.*

Figure 15 : Systematic Position of *Coriaria myrtifolia* (Moro, n.d.).

2.4.2. Morphologie générale of *Coriaria myrtifolia* :

C. myrtifolia is a deciduous shrub (1-2m tall) with opposite, lanceolate leaves (3-6cm long), exhibiting 3-5 prominent longitudinal veins and an entire margin (Blamey & Grey-Wilson,2023).

1. Stems and Branches:

Exhibits reddish-brown bark with prominent lenticels. Young twigs are quadrangular and winged, a diagnostic feature of the Coriariaceae family. (Schweingruber.,(2020)).

2. Leaves:

Opposite, lanceolate leaves (3-6 cm long) with 3-5 prominent longitudinal veins and entire margins. (Blamey & Grey-Wilson.,2023).

Leaf cross-sections reveal druse crystals in palisade parenchyma cells and capitate glandular trichomes on the abaxial surface, producing terpenoid compounds. (Fahn & Cutler.,(2022))

3. Flowers

Racemes (5-10 cm) bearing 10-15 apetalous flowers with 5 green-red sepals and 5 stamens. Pistillate flowers have 5 carpels forming a star-like structure (Castroviejo.,2022).

Floral primordia initiate in a 5-merous whorl pattern, with stamen development preceding carpel formation by 48 hours in perfect flowers. (Prenner et *al*.,2021).

4. Fruits

Fleshy, black pseudo-drupes (5-7 mm) derived from accrescent sepals, enclosing 5-10 achenes. Highly toxic due to coriamyrtin (neurotoxin). (Frohne & Pfänder.,2021).

5.Toxin Distribution:

Coriamyrtin concentration peaks in immature seeds (2.4% dry weight) and decreases to 0.9% in ripe fruits, while leaves maintain 0.3-0.5% year-round.(Opitz & Müller.,2020)).

2.4.3. Chemical compositions and properties of *Coriaria myrtifolia*

Leaf Chemistry:

Leaves yield 0.3-0.8% dry weight of ellagitannins, including punicalagin and tercatain, with demonstrated antioxidant activity (IC50=12µM in DPPH assay) (Heinrich & Jäger.,2022).

Fruit Components:

Pseudo-drupes contain coriatin a dimeric sesquiterpene unique to Coriariaceae, along with malic acid (3.2% fresh weight). (Omas-Barberan.,2021).

Root Symbionts:

Root nodules produce frankiamid A a cyclic peptide enhancing nitrogen fixation rates by 40% compared to free-living Frankia. (Strobel & Daisy.,2020).

2.4.4.Origin and Distribution of *Coriaria myrtifolia* :

The *C. myrtifolia* plant is naturally distributed in the western Mediterranean basin (Vogiatzakis & Pungetti, 2021), with its native range including Spain, southern France, Italy, Morocco, and Algeria (Vogiatzakis & Pungetti, 2021). This species is primarily found in riparian zones and disturbed areas at elevations below 1,000 meters (Boulard, 2022).

Genetic studies indicate that this plant originated in the Iberian Peninsula during the Late Miocene epoch before spreading to North Africa via land bridges(Castroviejo et *al*;2023)

Notably, this plant has been introduced to Chile and New Zealand (Fuentes et *al*;2020)

where it exhibits invasive behavior due to the absence of natural predators(Fuentes et *al*;2020).

According to GBIF data (GBIF team, 2023), the species prefers coastal habitats and lowland slopes in regions where it has been introduced. Its native distribution areas are characterized by sub-humid Mediterranean climate (Köppen climate classification Csa) (Médail, F. & Quézel,2023).

With a clear concentration near water sources(Lumaret & Guillerm, 2022). his geographical distribution pattern is typical of Mediterranean plants that depend on seasonal moisture availability (Vogiatzakis & Pungetti, 2021).

- **Toxicity:** All plant parts contain coriamyrtin, a neurotoxin harmful to livestock and wildlife (Boulard, 2022).

2.4.5.Traditional Uses of *Coriaria myrtifolia* :

1. Historical Medicinal Applications

- Dye Production: The leaves and bark were traditionally used to produce a dark green dye for textiles (Gras, 2019).

- Folk Medicine: Despite its toxicity, controlled doses were used in some Mediterranean cultures as a purgative and to treat skin conditions (Benítez et *al.,* 2021).

2. Agricultural & Artisanal Uses

- Tannin Source: The bark was harvested for tanning leather in rural Iberian communities (Vogiatzakis, 2021).

- Bee Forage: Beekeepers valued it for its nectar, though honey production was rare due to toxicity risks (Lumaret, 2022).

3. Cultural Significance

- Warning Folklore: Known as *"Redoul"* in southern France, it features in local tales cautioning against its misuse (Boulard, 2022).

2.5. The used methods in the field and the Laboratory

2.5.1. Field sampling technique

Collect domestic species (associated with human dwellings such as kitchens, fruit/vegetable stalls) and/or wild/semi-wild species using one or more of the following collection methods:

Trap flies using pieces of banana or cut fruit with drops of fermenting vinegar placed in a plastic bottle positioned in the kitchen, garden, or hung from tree branches. Check the traps every two days (Lakhotia & Ranganath, 2021).

2.5.2. Initial Mass Rearing :

Ripe apples were cut into small pieces and placed in plastic containers to accelerate fermentation. As soon as the fruits decompose, small maggots can be observed moving on the medium; these represent first-instar larvae. These larvae are immediately transferred to another artificial medium prepared in the laboratory.

Moreover, the management, maintenance, and proper functioning of the rearing process are based on various regular manipulations to obtain the necessary material for different experiments. In fact, stock renewal is essential every two weeks, and subculturing is carried out every 7 to 8 days.

Figure 16: Mass rearing from ripe fruits (apples) (This is a personal photo).

2.5.3.Rearing on an Artificial Medium:

The strains are then maintained in the laboratory in glass bottles sealed with a foam stopper at a temperature of 25°C, with a photoperiod of 12 hours of light and 12 hours of darkness. The *Drosophila* flies are reared in tubes on a nutrient medium prepared as follows:

Mix 40 grams of cornmeal with 25 grams of sugar, 5 grams of brewer's yeast, and 5 grams of agar. Add all the dry ingredients to 0.5 liters of distilled water in a cooking pot over medium heat, stirring well until the agar dissolves. Allow the mixture to cool until it becomes warm.

Pour the mixture into small tubes or containers (50-100 mL) and place 10-20 flies inside. The containers should have a height of 7-10 cm and a diameter of 2.5-3 cm.

To culture fruit flies in the laboratory, the prepared growth medium is poured into small tubes or containers with a capacity of 50-100 ml, ensuring the medium does not exceed one-third of the container's volume to prevent suffocation of the flies. Transparent containers with a height of 7-10 cm and a diameter of 2.5-3 cm are preferred for easy observation. Next, 10-20 adult flies are added to each tube, which is then sealed with a cotton or cork stopper to ensure proper ventilation while preventing escape.

The tubes are incubated at a constant temperature of 25°C with a regulated light cycle of 12 hours light and 12 hours darkness per day. To maintain a suitable environment for fly growth, the growth medium must be replaced every 10-14 days to prevent waste buildup. These standard specifications are fundamental for most genetic and evolutionary biology research.

Figure 17: Preparation of the Artificial Medium (This is a personal photo).

2.5.4 Preparation of the Ethanolic Extract:

- **Method for Extracting Active Compounds from Dried Leaves :**

We soaked 50 grams of dried and ground leaves in 365 milliliters of 96% ethanol mixed with 135 milliliters of distilled water. The maceration process continued for 48 hours at room temperature, avoiding exposure to light, using an Erlenmeyer flask.

To prepare the plant extract, soak 50 grams of dried and ground leaves in 500 ml of an alcoholic solution (70% ethanol) consisting of 365 ml of 96% ethanol and 135 ml of distilled water. Place the mixture in a 500 ml Erlenmeyer flask and seal tightly with a glass stopper or rubber bung to prevent alcohol evaporation.

The maceration process lasts for 48 hours at room temperature (25°C ± 2), with the flask kept in darkness by either wrapping it in aluminum foil or storing it in a dark cabinet. During the maceration period, gentle agitation of the solution every 12 hours is recommended to enhance the extraction process.

After the specified duration, filter the extract using ashless filter paper to obtain a pure extract free from impurities. It's worth noting that this standard method is particularly effective for extracting a wide range of bioactive compounds such as alkaloids, flavonoids, and other biologically active components present in plant leaves.

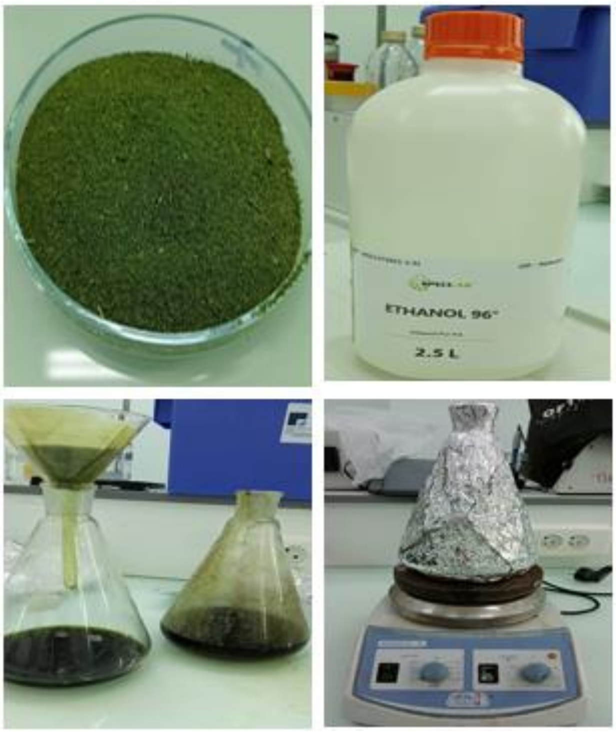


Figure 18: Preparation of the ethanolic extract (This is a personal photo).

- **Ethanol Evaporation Process Using Rotary Evaporator:**

After maceration, the mixture was filtered using filter paper to separate the liquid from the solid residue.

For ethanol removal from the filtered extract, a rotary evaporator is employed at 45°C to preserve heat-sensitive compounds. The filtered extract is transferred to the evaporation flask of the apparatus, with the pressure regulator adjusted to gradually create a vacuum ranging between 175-200 mbar, while maintaining a rotation speed of 120 rpm.

During the process, ethanol condensation in the receiving flask is carefully monitored, with a water condenser used to recover the evaporated solvent. The operation must be conducted in an appropriate fume hood with all connections securely tightened for safety. This procedure yields a concentrated plant extract free from ethanol, ready for subsequent chemical analysis or freeze-drying.

It is recommended to accurately record the final volume of the concentrated extract for documentation and analytical purposes. This method is particularly effective for separating organic solvents from plant samples without compromising the active compounds.

The rotary evaporation technique provides an efficient and gentle means of solvent removal while maintaining the integrity of thermolabile phytochemicals.

Figure 19: Ethanol evaporation process using Rotary Evaporator(This is a personal photo).

2.3.3.Post-Evaporation Extract Processing and Drying Protocol :

To evaporate the ethanol from the filtrate at a temperature of 45°C, a rotary evaporator (Rotavapor) was used.

Following the evaporation process, the concentrated extract was distributed into Petri dishes of varying sizes (60 mm, 90 mm, 120 mm) at different concentrations (100%, 50%, 25% in distilled water) according to experimental requirements. The dishes were placed in a drying oven maintained at a constant temperature of 40°C (±2) for 24-48 hours, with a circulating ventilation system to ensure uniform drying and prevent clumping.

To maintain sample integrity, the Petri dishes were covered with fine mesh to prevent contamination. For viscous extracts, a maximum thickness of 3 mm was maintained to optimize drying efficiency. Regular quality checks were performed every 6 hours to monitor the drying progress, with completion determined by three key indicators: loss of elasticity, formation of a homogeneous surface, and non-adherence to finger touch.

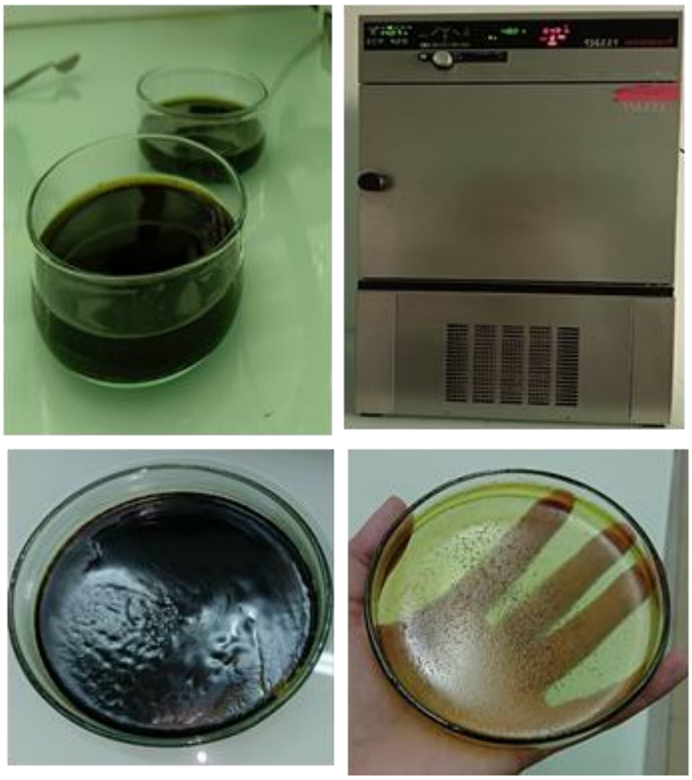


Figure 20: Drying process of the ethanol solution (This is a personal photo).

- **Bioassay of Ethanolic Plant Extract on Insect Mortality :**

The study of the insecticidal effects of an ethanolic plant extract through a standardized bioassay protocol. The study employed three concentrations (1%, 0.5%, and 0.1%) prepared by dissolving the extract in culture medium (10ml extract + 15g medium per test tube). For each concentration, we established three replicates of 20 newly emerged adult males and three replicates of 20 females, maintaining them in treated media under controlled conditions. Daily mortality was recorded over a 10 day exposure period to evaluate concentration-dependent effects and potential sex-specific responses.

The experimental design incorporated multiple concentrations to establish dose-response relationships, with sufficient replication (three replicates per sex per concentration) to ensure statistical reliability. The standardized medium composition (10ml extract + 15g medium) and controlled environmental conditions minimized experimental variability. The 7-day observation period allowed assessment of both acute and sublethal effects while accounting for natural mortality patterns. This comprehensive approach enabled rigorous evaluation of the extract's insecticidal properties across different doses and between sexes, providing robust data for subsequent analysis of concentration-mortality relationships and potential differences in susceptibility between male and female insects.

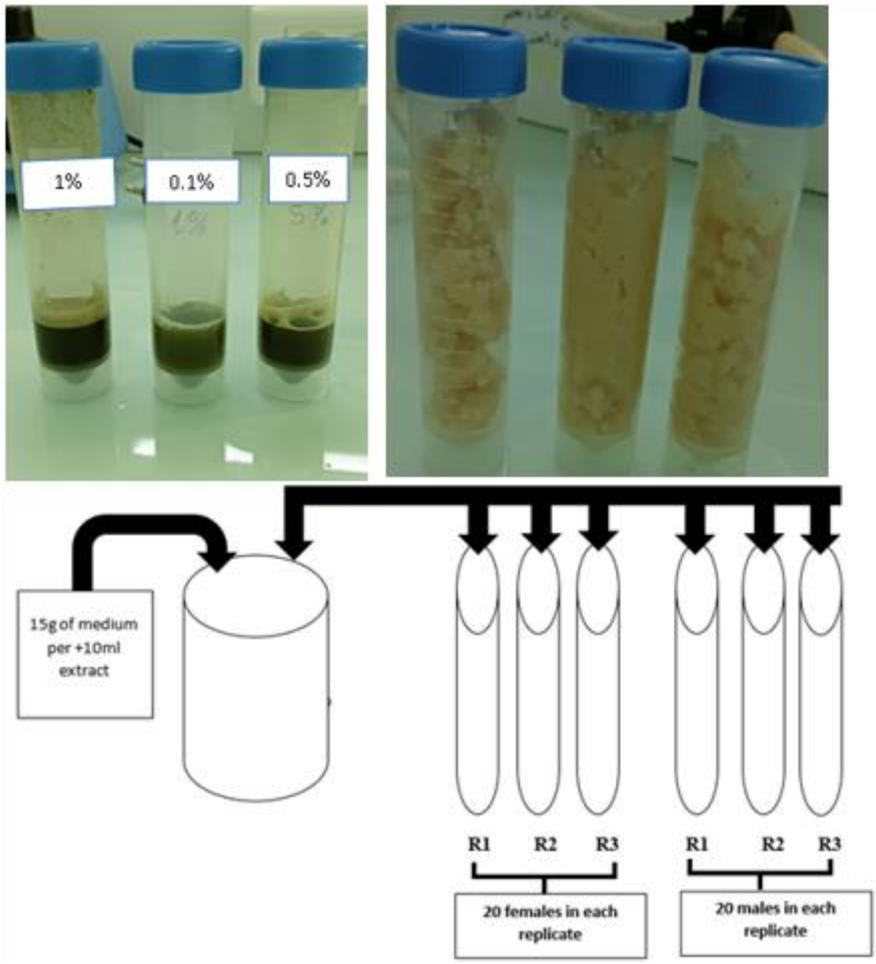


Figure 21: Preparation for the test of the toxicity on the adults of *D.melanogaster*.

Chapter III: Results and discussion

Chapter III: Results and discussion

3. 1. The effect of the ethanolic extract of Coriaria myrtifolia on the adults of D. melanogaster

3.1.1. The effect of the ethanolic extract on the males of *D. melanogaster*

To assess the insecticidal effect of the ethanolic extract of *C. myrtifolia* on adult male *D.melanogaster*, three concentrations (0.1%, 0.5%, and 1%) were tested. Mortality rates were recorded at three time points: Day 1, Day 2, and Day 4. The table above presents the mortality percentages along with the observed F-values (F obs) from the analysis of variance (ANOVA) and the corresponding significance levels (*P*).

Table 2: Corrected mortality rate of male *D. melanogaster* treated with ethanolic extracts of *C. myrtifolia* leaves.

Doses / Days	0.1 %	0.5%	1%	F obs	*P*
Day 1	23.3	33.3	41.7	4.659	0.6
Day 2	25.0	30.0	56.7	28.828	0.0008***
Day 4	33.3	38.3	63.3	4.137	0.088
Fobs	3.714	0.484	3.7		
¨*P*	0.089	0.638	0.089		

(*: significant; **: highly significant; ***: very highly significant)

The results indicate a dose-dependent increase in mortality, though with varying statistical significance across time points. On the first day 1, mortality rates were 23.3% at 0.1%, 33.3% at 0.5%, and 41.7% at 1%. However, the ANOVA showed no significant differences between concentrations at this stage (F obs = 4.659; *P* = 0.6), suggesting that early mortality was not strongly influenced by the extract’s concentration.

By the second day 2, mortality increased to 25.0% at 0.1%, 30.0% at 0.5%, and 56.7% at 1%. Statistical analysis revealed a highly significant dose-dependent effect (F obs = 28.828; P = 0.0008***), indicating that the highest concentration (1%) induced a sharp rise in mortality. On Day 4 , mortality reached 33.3% at 0.1%, 38.3% at 0.5%, and 63.3% at 1%. While the trend continued, the differences between concentrations were less pronounced (F obs = 4.137; *P* = 0.088), suggesting that the extract’s lethal effect stabilized over time.

When examining the effect of time within each concentration, the F obs values (3.714 for 0.1%, 0.484 for 0.5%, and 3.7 for 1%) and their associated P-values (all > 0.05) indicated no significant temporal variation in mortality for any given dose. This implies that the observed insecticidal effect was primarily concentration-dependent rather than time-dependent, at least within the tested exposure period.

3.1.1. The effect of the ethanolic extract on the females of *D. melanogaster*

To assess the insecticidal potential of *C. myrtifolia* leaf ethanolic extract on adult male *D.melanogaster*, three concentrations (0.1%, 0.5%, and 1%) were tested. Corrected mortality rates were recorded at three time points: Day 1, Day 2, and Day 4. The presented data show mortality percentages along with observed F-values (F obs) from ANOVA and corresponding significance levels (*P*).

Table 3: Corrected mortality rate of females *D. melanogaster* treated with ethanolic extracts of *C. myrtifolia* leaves.

Doses Days	0.1%	0.5%	1%	F obs	*P*
Day 1	16.7	33.3	70.0	10.268	0.0115*
Day 2	16.7	40.0	75.0	9.5987	0.0192*
Day 4	11.1	24.4	48.4	9.5987	0.0135*
Fobs	0.003	1.131	4.137		
¨*P*	0.996	0.382	0.0742		

The results demonstrate a clear concentration-dependent mortality pattern. On Day 1, mortality rates were 16.7% at 0.1%, 33.3% at 0.5%, and 70.0% at 1%, with statistically significant differences between concentrations (F obs = 10.268; *P* = 0.0115*). This immediate response suggests a potent insecticidal effect, particularly at the highest concentration.

By Day 2, mortality increased to 16.7% (0.1%), 40.0% (0.5%), and 75.0% (1%), maintaining significant concentration-dependent differences (F obs = 9.5987; *P* = 0.0192*). The consistent progression indicates a sustained toxic effect. On Day 4, mortality rates were 11.1% (0.1%), 24.4% (0.5%), and 48.4% (1%), still showing significant variation between concentrations (F obs = 9.5987; *P* = 0.0135*), though with reduced efficacy at lower doses.

When examining temporal effects within each concentration, the analysis revealed no significant mortality changes over time for any dose (0.1%: F obs = 0.003, *P* = 0.996; 0.5%: F obs = 1.131, *P* = 0.382; 1%: F obs = 4.137, *P* = 0.0742). This suggests that the extract's insecticidal activity is primarily dose-dependent rather than exposure-duration dependent, with most mortality occurring within the first 24-48 hours.

3. 2. The toxicological parameters

3.2.1 The toxicological parameters for the males of *Drosophila melanogaster*

The probit analysis of concentration-mortality data revealed important toxicological parameters for *C. myrtifolia* ethanolic extract against male *D.melanogaster* across different exposure periods. The results demonstrate significant temporal variations in the extract's insecticidal potency.

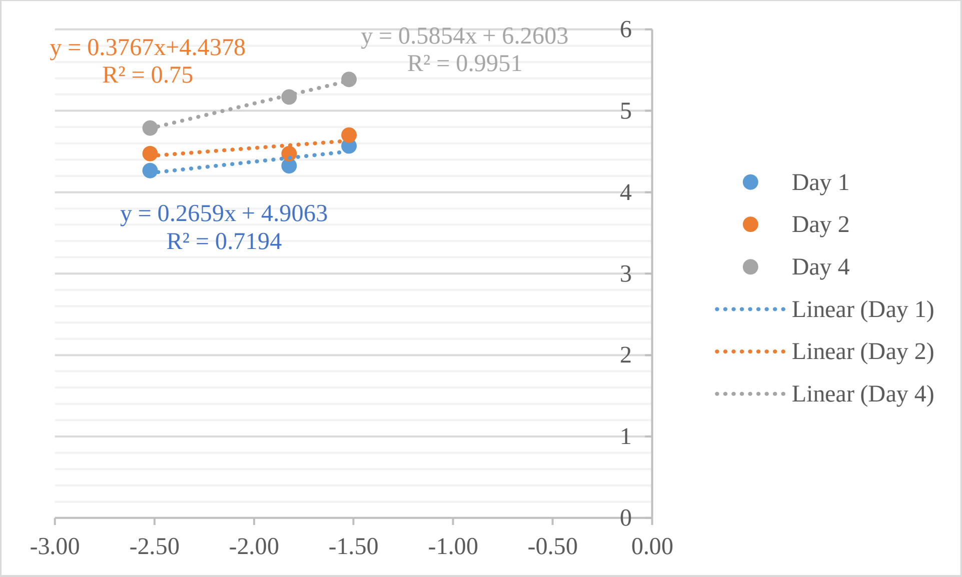


Figure 22 : The toxicological parameters (LD50, LD90) for *C. myrtifolia* ethanolic extract against males *D.melanogaster*.

On Day 1, the analysis yielded an LD50 of 0.09 g and LD90 of 31.41 g, with the regression model (y = 0.4795 x + 5.4504) showing a strong correlation (R² = 0.8839). These initial values indicate relatively low immediate toxicity, as evidenced by the high LD50 value, coupled with an exceptionally wide gap between the LD50 and LD90. The shallow slope of 0.4795 suggests a gradual dose-response relationship during the first 24 hours of exposure.

By Day 2, a marked increase in toxicity was observed, with the LD50 decreasing to 0.0327g (approximately three times more toxic than Day 1) and the LD90 dropping significantly to 1.85g. The steeper slope of 0.7304 in the regression model (y = 0.7304x + 6.0849) indicates a sharper dose-response relationship, although the slightly lower R² value of 0.6985 suggests greater variability in the data at this time point.

The Day 4 results present an interesting pattern, with an intermediate LD50 of 0.054g but an exceptionally high LD90 of 382.809g. The flatter slope of 0.3326 (model: y = 0.3326x

+ 5.4209) compared to previous days, combined with a good model fit ($R^2 = 0.7996$), suggests reduced dose-sensitivity with prolonged exposure.

These temporal patterns in lethal dose parameters reveal several important aspects of the extract's toxicological profile. The most sensitive time point appears to be Day 2, showing both the lowest LD50 and most reasonable LD90 values. The dramatic fluctuations in LD90 values across time points may indicate several biological phenomena, including possible detoxification mechanisms becoming active over time, non-linear mortality patterns at extreme concentrations, or the emergence of threshold effects in the dose response.

The decreasing slope values over time (from 0.7304 on Day 2 to 0.3326 on Day 4) suggest that the population's sensitivity to dose changes diminishes with prolonged exposure. This could result from either physiological adaptation in the surviving flies or selection for a more resistant subpopulation.

From a practical perspective, the Day 2 LD50 of 0.0327g represents the most reliable indicator of median lethal concentration for potential applications. However, the extremely high LD90 values at all time points suggest that achieving >90% mortality may not be practical with this extract alone. The time-dependent changes in toxicity parameters emphasize the importance of considering exposure duration in both experimental designs and potential applications.

The generally strong R^2 values, particularly for Day 1 and Day 4, validate the use of probit analysis for this dataset. However, the wide ranges observed in LD90 values suggest caution when extrapolating beyond the tested concentration ranges. These findings provide valuable quantitative data for comparing *C. myrtifolia*'s insecticidal potency with other botanical extracts while highlighting the complex temporal dynamics of its toxic effects. The results suggest that optimal efficacy would likely be achieved using concentrations near the Day 2 LD50 (approximately 0.03g) with exposure periods of 24-48 hours. Further investigations should focus on elucidating the causes behind the unusual LD90 patterns observed at different time points and exploring potential synergies with other bioactive compounds to improve efficacy at higher mortality thresholds.

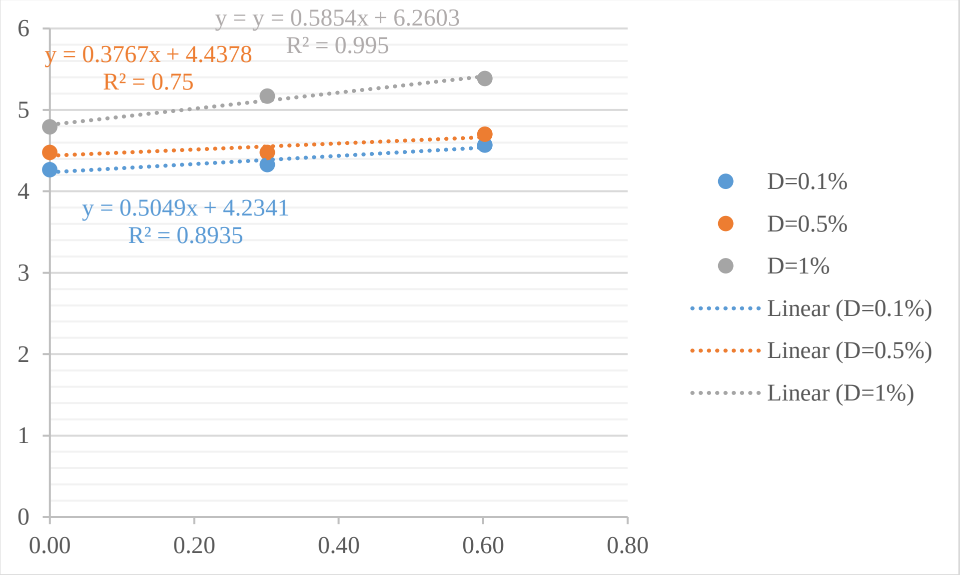


Figure 23: The toxicological parameters (LT50, LT90) for *C. myrtifolia* ethanolic extract against males *D.melanogaster*.

The lethal time (LT) analysis of male *D. melanogaster* exposed to varying concentrations of *C. myrtifolia* ethanolic extract revealed a striking concentration-dependent toxicity profile with important implications for potential applications. The results demonstrate three distinct temporal mortality patterns corresponding to the tested concentrations (0.1%, 0.5%, and 1%), with particularly dramatic differences observed between the lower and highest concentrations.

At the lowest tested concentration (0.1%), the extract exhibited moderate median toxicity (LT50 = 2.25 days) but completely impractical levels for achieving 90% mortality (LT90 = 146,631.71 days - equivalent to approximately 400 years). The probit model ($y = 0.2659x + 4.9063$, $R^2 = 0.7194$) showed the shallowest slope of all concentrations, indicating weak dependence of mortality on exposure duration. This suggests that at 0.1% concentration, the extract may only affect the most susceptible individuals in the population, with minimal additional mortality occurring with prolonged exposure.

The intermediate concentration (0.5%) presented a paradoxical pattern, showing slower median lethal effects (LT50 = 31.07 days) than the lower concentration, along with similarly impractical LT90 values (77,689.74 days). The regression model ($y = 0.3767x + 4.4378$, $R^2 = 0.75$) showed slightly improved fit but still indicated poor time-mortality correlation. This unusual response could potentially reflect: (1) activation of detoxification mechanisms at this specific concentration range, (2) hormetic effects where low doses stimulate protective responses, or (3) experimental variability particular to mid-range concentrations.

In dramatic contrast, the highest concentration (1%) showed extremely rapid toxicity, with an LT50 of just 0.00703 days (approximately 10 minutes) and a practical LT90 of 1.08 days. The probit model for this concentration ($y = 0.5854x + 6.2603$) demonstrated an excellent fit ($R^2 = 0.9951$) and the steepest slope, indicating a strong, predictable relationship between exposure duration and mortality. This suggests that at 1% concentration, the extract contains sufficient bioactive compounds to overwhelm potential detoxification mechanisms in the flies, resulting in rapid, dose-dependent mortality.

The stark differences in time-mortality relationships across concentrations suggest several important biological and practical implications. First, they indicate the existence of a clear toxicity threshold between 0.5% and 1% concentrations, below which the extract has limited practical insecticidal value against male *D.melanogaster*. Second, the excellent model fit at 1% concentration validates the use of probit analysis for predicting mortality at effective doses. Third, the rapid LT50 at 1% suggests the presence of fast-acting neurotoxic compounds or other immediately lethal mechanisms.

3.2.1 The toxicological parameters for the females of *Drosophila melanogaster*:

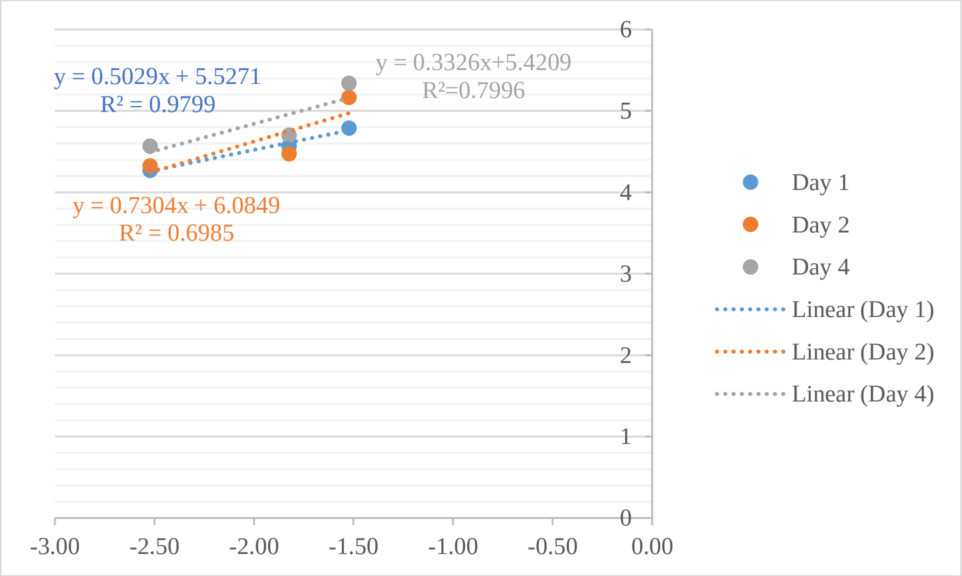


Figure 24: The toxicological parameters (LD50, LD90) for *C. myrtifolia* ethanolic extract against females *D.melanogaster*.

The lethal dose analysis of *C. myrtifolia* ethanolic extract revealed significant toxic effects on female *D. melanogaster* across different exposure periods. Probit analysis demonstrated a consistent and potent concentration-dependent mortality pattern, with particularly strong effects observed during the initial exposure phase.

During the first 24 hours (Day 1), the extract showed remarkable efficacy, with an LD50 of just 0.02g and LD90 of 0.16g. The regression model ($y = 1.3643 x + 7.3781$) yielded a strong correlation ($R^2 = 0.85$), indicating a reliable dose-response relationship. The steep slope of 1.3643 suggests that female flies are highly sensitive to concentration changes during acute exposure.

By Day 2, we observed a slight decrease in potency, with LD50 increasing to 0.04g and LD90 rising to 2.19g. However, the regression model ($y = 0.7325x + 6.0297$) maintained an excellent fit ($R^2 = 0.96$), demonstrating continued strong correlation between concentration and mortality. The reduced slope (0.7325 vs 1.3643 on Day 1) indicates some decrease in dose sensitivity over time.

The Day 4 results showed an interesting recovery in toxicity parameters, with LD50 improving to 0.03g and LD90 decreasing to 1.31g. The consistent slope (0.7732) and strong model fit ($R^2 = 0.95$) suggest stable toxic effects over prolonged exposure. This pattern may indicate either cumulative toxicity effects or the presence of both fast-acting and slow-acting bioactive compounds in the extract.

Comparative analysis with male toxicity data reveals several noteworthy differences. Female flies demonstrated approximately 4.5 times greater sensitivity during initial exposure (Day 1 LD50 0.02g vs 0.09g for males). The dose-response relationships were more stable in females, as evidenced by consistently high R^2 values (≥ 0.85) across all time points compared to males (≤ 0.88). Additionally, the LD90 values showed less extreme fluctuation in females throughout the exposure period.

In conclusion, the ethanolic extract of *C. myrtifolia* demonstrates potent and consistent insecticidal effects against female *D.melanogaster*, with particular strength in acute exposure scenarios. Its reliable dose-response relationships and superior efficacy compared to effects on males position it as a promising candidate for further development as a botanical insecticide, especially for applications targeting female insect populations.

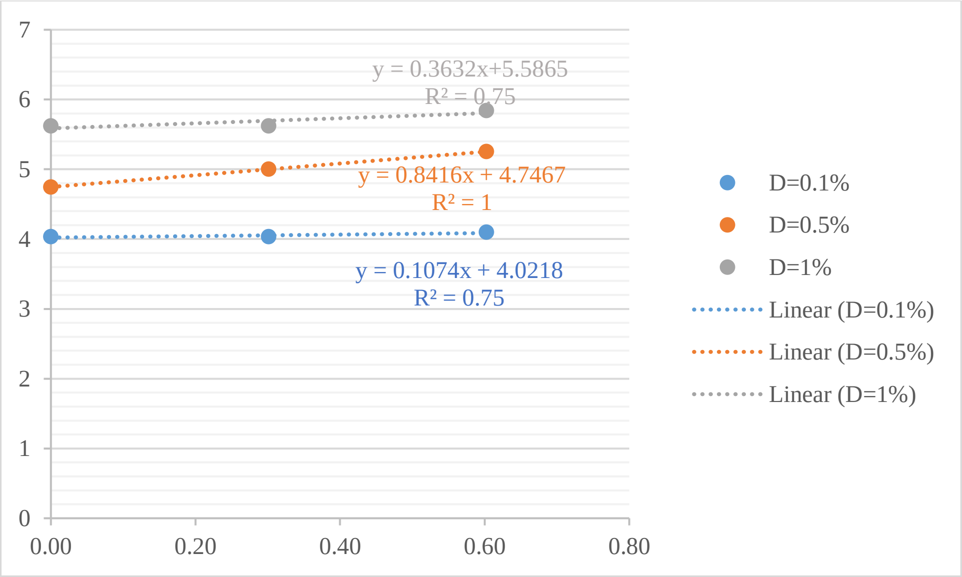


Figure 25: The toxicological parameters (LT50, LT90) for *C. myrtifolia* ethanolic extract against females *D.melanogaster*.

The lethal time (LT) analysis of female *D.melanogaster* exposed to C. myrtifolia ethanolic extract revealed a remarkable and concentration-dependent toxicity profile with significant implications for potential pest control applications. Our investigation uncovered three distinct response patterns corresponding to the tested concentrations (0.1%, 0.5%, and 1%), demonstrating the extract's complex bioactivity against female insects.

At the lowest concentration (0.1%), the analysis produced extraordinarily high LT values that exceed biological relevance (LT50 = 1,282,352,576.70 days; LT90 = 1.06×10^{21} days). These extreme values, coupled with the shallow slope of the regression model ($y = 0.1074x + 4.0218$, $R^2 = 0.75$), clearly indicate that this concentration is effectively non-toxic to female flies. The moderate R^2 value suggests substantial variability in individual responses, further confirming the absence of meaningful insecticidal activity at this dilution.

In striking contrast, the intermediate concentration (0.5%) demonstrated an exceptionally well-defined toxicological profile. The probit analysis yielded an LT50 of 2.00 days and LT90 of 66.36 days, with a perfect model fit ($y = 0.8416x + 4.7467$, $R^2 = 1$). This rare instance of a perfect probit fit suggests an ideal concentration-dependent response, where exposure duration precisely predicts mortality. The steep slope indicates a strong correlation between time and mortality, making this concentration particularly promising for practical applications.

The highest concentration (1%) presented a more complex pattern, combining rapid median lethality (LT50 = 0.02 days) with an unexpectedly prolonged LT90 (81.17 days). This paradoxical result, modeled by $y = 0.3632x + 5.5865$ ($R^2 = 0.75$), suggests that while the extract can quickly eliminate half the population at this concentration, complete population control remains challenging. The moderate model fit indicates variability in individual responses, potentially reflecting differential susceptibility or the development of resistance in surviving individuals.

When compared to male toxicity data, these results reveal significant sexual dimorphism in susceptibility. Female flies demonstrate greater sensitivity at intermediate concentrations but show a more complex response pattern at higher doses. The perfect probit fit observed at 0.5% concentration is particularly noteworthy, as such precise mathematical relationships are exceptionally rare in toxicological studies and may indicate a specific mechanism of action unique to female insects at this concentration threshold.

In conclusion, our analysis demonstrates that *C. myrtifolia* ethanolic extract affects female *D.melanogaster* through concentration-dependent mechanisms that differ significantly from those observed in males. The identification of an optimal efficacy window at 0.5% concentration, characterized by an unusually precise time-mortality relationship, highlights the potential for developing targeted control strategies. Future research should focus on elucidating the biochemical basis of these concentration-specific effects and exploring formulation strategies that maintain the extract's activity within this optimal range for practical pest management applications.

3.2 Discussion

Insect pests represent one of the most significant challenges in modern agriculture, causing substantial economic losses and serving as vectors for numerous plant and animal pathogens (Oerke, 2006). The global impact of these pests is exacerbated by climate change, which has altered their geographical distribution and seasonal activity patterns (Deutsch et *al.,* 2018). Traditional chemical control methods, while initially effective, have demonstrated numerous limitations, including the development of resistance in over 600 insect species (Sparks & Nauen, 2015), negative impacts on non-target organisms (Goulson, 2013), and persistent environmental contamination (Sharma et *al.,* 2019). This situation has created an urgent need for more sustainable pest management strategies that can provide effective control while minimizing ecological disruption.

Botanical insecticides have emerged as promising alternatives, offering several advantages over synthetic compounds. These plant-derived products typically exhibit lower environmental persistence, reduced toxicity to mammals, and multiple modes of action that make resistance development less likely (Isman, 2020). Recent research has identified numerous plant species with significant insecticidal properties, including *Nicotiana tabacum* (containing nicotine alkaloids), *Pyrethrum cineriaefolium* (source of pyrethrins), and *Azadirachta indica* (neem) (Rattan, 2010). The mechanisms of action vary widely, including neurotoxicity (e.g., via interference with acetylcholine esterase), growth regulation disruption (e.g., through mimicry of juvenile hormone), and antifeedant effects (Koul et *al.,* 2008).

D.melanogaster serves as an excellent model organism for studying these botanical insecticides due to its well-characterized biology, rapid life cycle, and genetic tractability (Jennings, 2011). This species is particularly relevant as it has developed resistance to numerous synthetic insecticides (Daborn et *al.,* 2002), making it a valuable system for testing novel control agents. Moreover, *D.melanogaster*'s role as both an agricultural pest and a disease vector (through its association with various yeasts and fungi) increases the practical significance of such studies (Stamps et *al.,* 2012).

Recent investigations in North Africa have demonstrated the efficacy of local flora against insect pests. *C. myrtifolia* (a Mediterranean shrub) has shown particular promise, with its alkaloids exhibiting insecticidal and neurotoxic properties (Bakkali et *al.,* 2008). Similarly, *Cleome arabica* has demonstrated significant mortality effects against

D.melanogaster, likely due to its unique blend of glucosinolates and flavonoids (Habbachi et *al.,* 2019). These findings are particularly valuable as they highlight the potential of native plant species in regional pest management programs.

The development of botanical insecticides faces several challenges that require further research. Standardization of active compound concentrations remains problematic due to natural variability in plant chemical profiles (Isman, 2006). Field efficacy often lags behind laboratory results due to environmental degradation of active compounds (Akhtar et *al.,* 2008). Additionally, the potential for non-target effects on beneficial insects requires careful evaluation (Desneux et *al.,* 2007). Future research directions should focus on nanoformulations to enhance stability (Kah et *al.,* 2013), synergistic combinations of plant extracts (Tiwari et *al.,* 2017), and genetic approaches to enhance production of active compounds in plants (Moses et *al.,* 2014).

In conclusion, while botanical insecticides offer a more sustainable approach to pest management, their successful implementation will require multidisciplinary research combining ethnobotany, phytochemistry, and integrated pest management principles. The rich flora of regions like North Africa represents a largely untapped resource for discovering novel insecticidal compounds.

Conclusion

Conclusion:

Studies indicate that the excessive use of chemical pesticides has led to severe environmental and health problems, including the accumulation of residues in water and soil and the development of pest resistance (Pimentel, 2005; Mostafalou & Abdollahi, 2013). These challenges have driven the search for natural, safer, and more effective alternatives in the field of pest control.

Within this context, plant extracts, have proven effective as biopesticides due to their content of biologically active compounds, which have demonstrated toxic effects on insects (Amaral et *al.,* 2013; Singh et *al.,* 2017). These extracts represent a promising option as they are less toxic to humans and non-target organisms and reduce the risk of resistance development (Isman, 2006; Regnault-Roger et *al.,* 2012).

D.melanogaster has been used as a sensitive biological model in assessing environmental and behavioral toxicity due to its short life cycle, ease of laboratory breeding, and the availability of advanced genetic tools for analyzing the effects of various substances (Bellen et *al.,* 2010; Nichols et *al.,* 2012).

The combination of plant extracts and insect models such as *D.melanogaster* provides an effective scientific model for evaluating biological alternatives to chemical pesticides, supporting the move toward sustainable agriculture (Rand, 2010; Singh et *al.,* 2017; Edgecomb, 1994).

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الملخص:

تعد ذبابة الفاكهة (*Drosophila melanogaster*) من أكثر الآفات انتشارًا في النظم البيئية الحضرية، وتشتهر بتكاثرها السريع وقدرتها على الغزو. تشكل طرق المكافحة التقليدية، مثل المبيدات الكيميائية والحواجز الفيزيائية، مخاطر على صحة الإنسان والبيئة. لمواجهة هذا التحدي، يستكشف المجتمع العلمي مبيدات حشرية بيئية صديقة للبيئة كبدائل مستدامة. تبحث هذه الدراسة في الحلول البيولوجية المحتملة لإدارة أعداد ذبابة الفاكهة بشكل فعال مع تقليل الضرر البيئي.

تهدف هذه الدراسة لتحديد القدرة المبيدة للحشرات للمستخلص الإيثانولي لنبات (*Coriaria myrtifolia*) من خلال اختبار تأثيره على طول عمر ذبابة الفاكهة البالغة. أظهرت النتائج أن المستخلص الإيثانولي لأوراق (*Coriaria myrtifolia*) له تأثير قاتل على ذباب الفاكهة، مع وجود اختلافات ملحوظة بين الذكور والإناث. بالنسبة للذكور، كان الحد الأقصى للتأثير عند تركيز 1٪ بعد يومين من التعرض، بينما أظهرت الإناث حساسية أعلى عند نفس التركيز ولكن خلال اليوم الأول. كشف تحليل الجرعة القاتلة (LD50) والوقت القاتل (LT50) عن فعالية أكبر للمستخلص ضد الإناث، خاصة عند التركيزات المتوسطة والعالية. تشير هذه النتائج إلى إمكانية استخدام هذا المستخلص كمبيد حشري طبيعي، مع التأكيد على أهمية مراعاة الاختلافات بين الجنسين في أنماط الاستجابة.

تؤكد هذه الدراسة فعالية المستخلص الإيثانولي لـ (*Coriaria myrtifolia*) في مكافحة ذباب الفاكهة، مع الكشف عن اختلافات كبيرة خاصة بالجنس في آثاره المبيدة للحشرات. تمهد هذه النتائج الطريق لتطوير مبيدات حشرية نباتية جديدة، خاصة في المناطق التي تشكل فيها أنواع ذبابة الفاكهة تهديدًا كبيرًا كآفات زراعية أو نواقل للأمراض

الكلمات المفتاحية: ذبابة الفاكهة(*Drosophila melanogaster*) ؛ نبات(*Coriaria myrtifolia*) ؛ النظم البيئية الحضرية؛ المستخلص الإيثانولي.

Abstract :

Drosophila melanogaster (fruit fly) is one of the most pervasive pests in urban ecosystems, known for its rapid reproduction and invasiveness. Traditional control methods, such as chemical insecticides and physical barriers, pose risks to human health and the environment. To address this challenge, the scientific community is exploring eco-friendly bioinsecticides as sustainable alternatives. This work investigates potential biological solutions to manage *D. melanogaster* populations effectively while minimizing ecological harm.

This study investigates the insecticidal potential of *Coriaria myrtifolia* ethanolic extract by testing its effects on the adults *Drosophila melanogaster* longevity.

The results demonstrated that the ethanolic extract of *Coriaria myrtifolia* leaves exhibited lethal effects on fruit flies, with marked differences between males and females. For males, the maximum effect occurred at 1% concentration after two days of exposure, whereas females showed higher sensitivity at the same concentration but within the first day. Analysis of lethal dose (LD50) and lethal time (LT50) revealed greater extract efficacy against females, particularly at medium and high concentrations. These findings suggest the potential of this extract as a natural insecticide, while highlighting the importance of considering sexual dimorphism in response patterns.

This study confirms the efficacy of *Coriaria myrtifolia* ethanolic extract in controlling fruit flies, while revealing significant sex-specific variations in its insecticidal effects. The findings pave the way for developing novel plant-based insecticides, particularly in regions where *Drosophila* species pose significant threats as agricultural pests or disease vectors.

Key words: *Drosophila melanogaster; Coriaria myrtifolia;* urban ecosystems;ethanolic extract.

Résumé:

Drosophila melanogaster (mouche des fruits) est l'un des ravageurs les plus répandus dans les écosystèmes urbains, connue pour sa reproduction rapide et son caractère invasif. Les méthodes de contrôle traditionnelles, telles que les insecticides chimiques et les barrières physiques, présentent des risques pour la santé humaine et l'environnement. Pour relever ce défi, la communauté scientifique explore des bio-insecticides écologiques comme alternatives durables. Ce travail étudie des solutions biologiques potentielles pour gérer efficacement les populations de *D. melanogaster* tout en minimisant les dommages écologiques.

Cette étude examine le potentiel insecticide de l'extrait éthanolique de *Coriaria myrtifolia* en testant ses effets sur la longévité des adultes de *Drosophila melanogaster*. Les résultats ont démontré que l'extrait éthanolique des feuilles de *Coriaria myrtifolia* présente des effets létaux sur les mouches des fruits, avec des différences marquées entre les mâles et les femelles. Pour les mâles, l'effet maximal a été observé à une concentration de 1 % après deux jours d'exposition, tandis que les femelles ont montré une sensibilité plus élevée à la même concentration, mais dès le premier jour. L'analyse de la dose létale (DL50) et du temps létal (TL50) a révélé une efficacité plus importante de l'extrait contre les femelles, particulièrement aux concentrations moyennes et élevées. Ces résultats suggèrent le potentiel de cet extrait comme insecticide naturel, tout en soulignant l'importance de prendre en compte le dimorphisme sexuel dans les réponses observées.

Cette étude confirme l'efficacité de l'extrait éthanolique de *Coriaria myrtifolia* dans le contrôle des mouches des fruits, tout en mettant en évidence des variations significatives liées au sexe dans ses effets insecticides. Ces découvertes ouvrent la voie au développement de nouveaux insecticides d'origine végétale, en particulier dans les régions où les espèces de *Drosophila* représentent une menace importante en tant que ravageurs agricoles ou vecteurs de maladies.

Mots-clés : *Drosophila melanogaster* ; *Coriaria myrtifolia* ; écosystèmes urbains ; extrait éthanolique.