

**Ceratitis capitata Wiedemann (Diptera: Tephritidae) in Moroccan Citrus orchards: Chemical and Sustainable Control Methods and Priorities for Future Research**Amine Assouguem^{1*}, Mohammed Kara², Safaâ Benmessaoud², Hamza Mechchate³, Zineb El Jabboury¹, Abdellah Farah⁴, Abderrahim Lazraq¹¹Laboratory of Functional Ecology and Genie of Environment, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, Imouzzer Street, Fez, P.O. Box 2202, Morocco²Laboratory of Biotechnology, Conservation and Valorisation of Natural Resources (LBCVNR), Department of Biology, Sidi Mohamed Ben Abdellah University, Faculty of Science Dhar El Mahraz, Fez, Atlas, Morocco³Laboratory of Biotechnology, Environment, Agri-Food, and Health (LBEAS), Faculty of Science Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez, BP 1796, Morocco⁴Laboratory of Applied Organic Chemistry, Faculty of Sciences and Technology, Sidi Mohamed Ben Abdellah University, Imouzzer Street, Fez, P.O. Box 2202, Morocco

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ABSTRACT

The Mediterranean fruit fly (*Ceratitis capitata*) infects fruits from over 300 plant species, causing substantial economic losses amounting to millions of dollars annually. Though it has its origin in the Mediterranean basin, it has spread to Asia, Europe, Central and South America, Africa, Australia, etc. Its control in Morocco has been principally based on chemical sprays.

The review aims to provide information on control methods of *Ceratitis capitata*, mainly in citrus orchards in Morocco. Relevant literature was sourced from different search engines: Google scholar, science direct, PubMed, etc. The article highlighted the principal findings of previous studies, discussed current control methods, and proposed new innovative research and sustainable approaches to control medflies.

Setting up an integrated long-term ecological monitoring program in Morocco and different African countries and incorporating new technologies into monitoring programs are urgently needed to fight this devastating pest. These programs must consider the climate conditions, phenology, infestation rates, and propagation speed of pests. Other methods include new organic pests and trapping, sterile insect technique (SIT), parasitoid enemies such as *Diachasmimorpha longicaudata*, pathogens like *Beauveria bassiana*, and introducing a self-limiting genetic method. These fundamental elements are suggested to control pests, respect the environment and public health.

Keywords: *Ceratitis capitata*, Citrus orchards, *Diachasmimorpha longicaudata*, Medflies, Morocco.

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Introduction

In Morocco, the growing area of citrus lands has exceeded 130,000 ha with a total production of more than two million tons, thanks to strategies put in place by the Ministry of Agriculture.¹ Moreover, government policy has projected an estimated production output between 2.5 and 3.5 million tons before 2022. The extension of production to other areas is also targeted.²

The citrus fruit is mainly cultivated in Moulouya, Souss, Tadla, Haouz Loukkos, and Gharb regions.^{1,2} Currently, there is an extraordinary change in the national citrus market, with a significant decrease in exports to the European Union from 70% to only 25-30%.³ In comparison, exports to Russia have increased enormously from 45 to 50% of all Moroccan exports.⁴ Unfortunately, shortage of water and intense competition in the international citrus market constitutes the

main constraints threatening the citrus sector in Morocco.^{5,6}

Unlike recent climatic challenges, pest control management is a significant and old constraint for citrus exports due to the international market's demand for quality. The steady supply of quality fruits without pest infestation, damage, or pesticide residues, requires sustainable citrus pest approaches.⁷ For citrus fruits, many pests cause an essential loss in quality and quantity: fruit fly *C. capitata* Wiedemann (Diptera: Tephritidae), red mite *Panonychus citri* McGregor (Acari: Tetranychidae), California red scale *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), and the citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae).⁸⁻¹⁰ Currently, other pests, including the two-spotted mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and the citrus brown mite *Eutetranychus orientalis* Klein (Acari: Tetranychidae), have also been recorded to impact citrus production considerably.¹¹ Among the citrus pests, the medfly is undoubtedly the most destructive pest, with indirect and direct economic effects.¹²⁻¹⁴ Despite control measures, 10 to 20% of production is regularly infested.¹⁵ Farmers are required to monitor medfly prevalence during the fruit-maturation phases, carefully and potentially apply several pesticide sprays to control this insect pest.¹⁶ Furthermore, to export fruit to countries like the USA, UK, and Japan, exporting countries need cold treatment conditions as quarantine procedures for pests of medfly, which raises costs.¹⁷ Although enormous efforts were made to control Morocco's medfly, this pest is still a significant menace to the entire national citrus industry (exports and production).¹⁸ For example, because of medfly

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infestation, the Department of Agriculture's Animal and Plant Health Inspection Service had to temporarily forbid importing some products, including clementines, tangerines, mandarins, and sweet oranges produced or shipped from the Berkane region during 2016.¹⁹

The review aimed at two objectives, namely: summarize existing literature on current approaches used to control the medfly population, prompt the employment of integrated medfly-management techniques based on environmentally-friendly practices, such as biological control, biopesticides, or the use of a self-limiting genetic process as well as discuss high-priority research guidelines to investigate new sustainable management strategies that have been tested in other countries with similar contexts.

Materials and Methods

The authors collected the different information and control methods used on *C. capitata* from articles cited in indexed journals, from Google Scholar, Science Direct, PubMed, Web of Science, MDPI, etc., using different search criteria such as biopesticides, *C. Capitata*, medfly, pest control, and management.

Results and Discussion

Status of *C. capitata* in Morocco

In Morocco, as in other Mediterranean countries, the medfly (Mediterranean fruit fly) *C. capitata* is considered the most abundant tephritid pest and constitutes the most damaging fruit pests with a severe threat to fruit cultivation, production, and export.²⁰ It is closely related to suitable host plants, fruit availability, and habitats features, including environmental conditions.²¹ Outside orchards, forest woodlands such as the Indian fig opuntia (*Opuntia ficus-indica*) and *Argania spinosa* offer huge and potential reservoirs of medflies for nearby citrus growing areas principally in the Souss region, where production accounts for 50% of the Moroccan production.^{22,23} The medflies affect other fruit plantations like apples, pears, nectarines, peaches, and plums pomegranates, in different regions and environmental conditions.^{24,25}

Control approaches in Morocco

In Morocco, pest management is profoundly dependent on chemical treatments.²⁶ Before 2010, the use of sustainable strategies was neglected and rudimentary. However, the application of eco-friendly methods has recently been slowly adopted due to public concerns (local and international markets) over chemical compounds, residues, and pest resistance to pesticides.²⁷ Several eco-friendly methods have revealed efficiency in the control of this pest. These methods could be utilized as control measures to meet the new international market requirements regarding fruit protection, quality, healthy environment, and human health.^{28,29} —Consequently, sustainable approaches to chemical control like biopesticides,^{30,31} the sterile insect technique (SIT),¹⁹ cold treatment³² and mass trapping³³ are used. Other methods, such as the continued release of self-limiting medfly strains, are also being studied.^{3,4}

Unsustainable control

Generally, unsustainable methods are based on chemical and synthetic substances, including pesticides, insecticides, fungicides, nematicides, and herbicides.³⁵ In Morocco, chemical control is the most extensively used approach against medflies and other pests. Usually, treatments are used with a full-cover spray (with non-professional stuff) and a wide range of significant spectrum pesticides, which are incredibly destructive to natural pest enemies (beneficial insects, birds, bats, etc.).³⁶ Instead, the foliar spot's treatments using hydrolysed protein baits contain the other leading chemical application practice recently used.³⁷ Equally, baits are based on bio-ecology, diurnal and seasonal behaviour of medflies.³⁸ In reality, ingestion of proteins during the maturity (adult) phase improves male fitness via pheromone production, leks, the rate of sexual intercourse, and the rate of sperms transmission.^{39,40}

Similarly, protein is also essential for egg production in medfly females.⁴¹ Rotten fruit, bird droppings, fungi, and bacteria are natural sources of proteins.⁴² Bait sprays consist of a protein source to attract adult medflies and an insecticide to kill them. By conjoining both methods in one invention, the bait is attractive as a nutrition source to both female and male medflies, which feed over the leaves for nutriment as approved currently.⁴³

The "Spinosad" compound has become the favoured chemical treatment, particularly during the harvest phase, because of its short persistence in the ecosystem and its average pre-harvest interval of one day.⁴⁴ Infestation rate and adult pest prevalence fruit define the date and regularity of chemical sprays.⁴⁵ Therefore, the monitoring starting point that designates when spraying should start is three to five adult males per trap and day.⁴⁴ On the other hand, in small orchards, sprays are applied once every ten days, mainly during late summer and autumn periods for the precocious citrus varieties and between the end of winter and spring, for the tardive varieties, depending on the yield zone.¹ However, to conform with Russian quarantine protocols of imported crops, an arrangement has been engaged with Russia targeting to support medfly control by farmers interested in the Russian market, reducing the chemical spray threshold to 1 male or 0.5 females/(trap*day).

Sustainable approaches

Biological control

Generally, all citrus-growing regions have the following main pests; fruit flies, armoured scales, mealy bugs, mites, and thrips.^{46,47} Most of these insect or mite pests are vulnerable to attack by natural enemies (entomopathogens). Then citrus pests must be seen as potential targets and explored.^{48,49} Among the medfly's natural entomopathogens, we list parasitoids (mainly from the Braconidae family), the most regularly used in biological pest control.⁵⁰ However, recently, an enormous number of parasitoids such as the larval-pupal parasitoids *Fopius vandenboschi*, the egg-pupal parasitoid *Fopius arisanus*, *Aganaspis daci*, *Diachasmimorpha tryoni*, *Diachasmimorpha longicaudata* and *Psytalia incisi* have been approved as significant enemies to the medfly inside citrus orchards.⁵¹ Several pilot studies were performed in Spain to evaluate the capacity of *Diachasmimorpha longicaudata* and *Aganaspis daci* for the medfly's biological control. The results showed that the parasitoids could disperse and parasitize medfly larvae and eggs in both laboratory and greenhouse.⁵¹ With the similarity in climate condition, agricultural practices between Morocco and Spain, these parasitoids are good biological enemies for Morocco testing.²⁵ However, research needs to investigate the new potential and indigenous parasitoids and natural enemies to control medfly and other destructive pests.

Use of biopesticides

The demand for biopesticides in agriculture is increasing due to the need to limit hazardous chemical pesticides.⁵² In this context, the close alternative uses pesticide substances derived from biological materials with selective action against target pests and less persistence in ecosystems.

Although farmers may control medflies through chemical use in Morocco, awareness of their destructive impacts is rising.²⁵ Therefore, biopesticides like *Beauveria bassiana*, Azadirachtin and Spinosad have been listed for use.⁵³ These active substances are confirmed to have low persistence and limited side impacts on non-target insects. Besides, other natural materials can be extracted from Moroccan ecosystems. Currently, bacteria such as *Bacillus thuringiensis* and *Fusarium* as a biopesticide are essentially among the best alternatives to chemicals for controlling insect agricultural pests.⁵⁴

Recent investigations proposed that the soil from the Argan forests (from the Souss region) may be a promoting reservoir for future and more comprehensive screening programs to identify microorganisms that can effectively control pests.⁵⁴ It was previously published that most autochthonous strains of *Beauveria bassiana* isolated from *Argania Spinosa* endemic species to Morocco were highly infectious to medfly.²⁵ Therefore, these indigenous isolates might be a source of potential biological control for medfly.

Sterile insect technique

Sterile insect technique was developed during the last century as a promoting technology for pest control.⁵⁵ The technique has progressively been viewed as an appropriate method for integrated pest management (IPM) for certain agricultural pests due to the growth of restrictions on the use of chemicals and the increasing demand for organic products.⁵⁶ Similarly, SIT is used in Europe (Portugal, Spain), Asia (Israel, Jordan), Africa (South Africa, Tunisia), North (USA, Mexico), and South America (Chilie, Argentina) in several medfly programs as the primary control strategy.⁵⁷

In Morocco, an agreement was signed by the citrus farmers and the Ministry of Agriculture to establish an area-wide sterile insect technique program to control the Mediterranean fruit fly in the Agadir region.^{55,58} The pilot project was carried out to defeat medflies in approximately 5,000 ha in the Souss Valley, using an SIT component. Eight million sterile male flies were released weekly. In 2014, the Ministry of Agriculture ordered to build a medfly mass-rearing infrastructure and a sterile male-release station, with technical assistance from FAO/IAEA (Food and Agriculture Organisation /International Atomic Energy Agency), in partnership with Moroccan citrus. The constructions were enough to offer sterile males to cover the entire Souss area with an estimated surface of 40,000 ha of citrus-producing zones and the neighbouring regions. The facility is expected to produce between 130 to 200 million sterile males per week.⁵⁶ However, field evaluations' efficiency is still ongoing before the enlargement to cover all the Souss zone. The application of SIT as a nationwide program (All national zones producing citrus) is the targeted objective. As a first evaluation, after three years of release, despite encouraging environmental conditions for medflies between 2010 and 2011, the abundance of female medflies in the examined zone declined compared to previously recorded levels. Consequently, chemical sprays were decreased by 25-75% alongside this approach.⁵⁶ Generally, the SIT centres' achievement for rearing and release will guarantee highly cost-effective medfly control levels and cover all citrus-growing zones.

Mass trapping

Mass trapping is a sustainable and ecologically friendly insect control technique founded on the use of species-specific synthetic chemical traps, such as aggregation pheromones and sex or food/host attractants.⁵⁹

Medfly mass trapping has been widely applied in Mediterranean countries by developing an artificial food-based female attractant: An extremely selective and potent combination of three chemical compounds produced for attracting female medflies.¹⁹ It has proven to be very effective in managing *C. capitata*, *T. absoluta*, and its application in the Mediterranean zone has augmented remarkably.⁵⁹ In Morocco, various synthetic attractants, which are easy to handle and have a long life span, have been developed to substitute protein solutions. Putrescine, trimethylamine hydrochloride and ammonium acetate are the most excellent examples of such attractants. They are combined in one formula or used separately depending on the efficiency of each attractant. These materials are highly female selective and have a prolonged action period of at least 90 days.⁴⁴ In Moroccan farmlands, the application of attractant and killer technics implicates using the M3 bait station (blue plastic trap saturated with alpha-cypermethrin and protein hydrolysate, etc.) is abundant and very efficient, permitting the avoidance of spray treatments in stations where M3 was set.¹ However, the application of mass trapping in Morocco separately or as part of an integrated medfly pest control permits the decrease of chemical overuse.¹⁹ Therefore, environmental pollution, pest proliferation, and chemical residues are minimized in the citrus production sector.

Cold treatment

The medfly *C. capitata* belongs to a list of pests with the maximum possibility of introducing pest-free provinces, countries, or areas.⁶⁰ Generally, to avoid the risk of disease and accidental introduction of invasive insect pests from imported fruit, many citrus fruit-importing countries request quarantine protocols, such as the case of Japan and the USA.¹⁹ In Morocco and all Mediterranean basin countries, citrus

farmers need to apply quarantine treatments before exportation dates.⁶¹ The quarantine treatment is 16 days at temperatures between 0 to 2°C. However, this technique is expensive and has other disadvantages, mainly the vulnerability of some varieties (tangerines and clementines) to low temperatures⁶³ and the difficulty of reaching far markets due to long quarantine period.

Morocco's citrus exported to the US market increased from 7.800 t in 2006/2007 to 42,011 t in the marketing year 2013/2014. Morocco was required to send these cargos via Europe for cold treatments.^{19,63} The shortest shipment to the US and the implementation of the Morocco-U.S Free Trade Arrangement were expected to increase citrus exports to the US in the near future.^{25,64} Equally, exports to China involve quarantine treatment for various pests. Therefore, Moroccan citrus fruit destined for this vast Asian market must be exposed to a minimum temperature of 1°C for 16 days.⁶⁵

However, to involve other international markets, new cold-treatment experimental protocols and technologies are required to open new windows for Moroccan products and increase revenues.⁶⁵ For example, Japan is a promising market for numerous Moroccan agricultural products. Morocco has signed an agreement between Maroc Citrus, Agronomic and Veterinary Institute (IAV) Hassan II, National Office of Food Safety (ONSSA), and the SAOAS Company to apply a cold-treatment experimental protocol delivered by Japanese consultants. The study's main goal was to verify the efficacy of cold treatment on infected fruit and fell within the overall strategic objective of eliminating the risk of the Mediterranean fruit fly introduction to Japan ecosystems.¹⁹

Sustained release of self-limiting medflies

Improved sterile pests are another possible technique to moderate the expensive costs of SIT-like programs and increase efficiency.^{34,66} The SIT is a mating-based method whereby released sterile males (sterilized via irradiation) compete with their wild competitors for successful mating with wild females inside orchards.⁶⁶ Compared to wild males, the exposure of males to irradiation causes a complete loss of competitive mating aptitude.⁶⁷ In the field, treated insects (with irradiation) have reduced life cycles and are less competitive (mainly in mating).⁶⁸ Consequently, fitness is reduced by 4-10-fold for medflies.^{69,70} To address the limitations of existing SIT approaches, the self-limiting technology is a novel and improved alternative, which aims specifically the expensive filter colony necessary for the stability of temperature-sensitive lethal mutation medfly strains caused by the use of irradiation and the uselessness of fluorescent powders. Indeed, sexing sterile males via genetics can be more effective than releases including mixed-sex populations.⁷¹ In summary, the self-limiting method offers novel operational genetic sexing instead of the expensive and performance-reducing method of sterilization using irradiation. Similarly, fluorescent marking permits easy distinguishing between transgenic and wild insects in both laboratory and field, eliminating the use of fluorescent powders, which can damage specimens.

Future research directions

Investigations on medfly

Determining spatiotemporal variation of population size, details of the biological cycle, and ecological features of medflies in Moroccan orchards are paramount to improving our understanding of the population dynamics in each region and climate conditions. Similarly, micro-conditions should be addressed carefully to clarify the whole picture of medflies inside orchards. Understanding life-cycle details and surrounding conditions will help identify the vulnerable stage that farmers could attack and the proper periods for treatment. Equally, the precise life cycle knowledge will help develop biopesticides and natural enemies that will disturb the normal medfly cycle with high effectiveness. Assessing the relationship between climatic variables (precipitation, temperature, humidity, and wind speed) and the medfly's presence, abundance, and reproductive success is essential to guiding control actions and adopting appropriate future management. Additional investigations on *C. capitata* feeding behaviour and population genetic structures are also crucial for any program's success.²⁵

Investigations on new control methods

Research into new pest management programs is urgently needed. This program should incorporate new materials such as extracts from aromatic and medicinal plants. These materials have shown promising results in pest control, including medfly.⁵² In parallel, natural enemies need to be investigated deeply because of their direct effect on pest insects. Remarkably, these entomopathogens are suggested to be well adapted inside orchards.

Conclusion

The study reviewed and highlighted the existing knowledge and future perspective of medfly control approaches used in Morocco. The prospective programs that can be used to manage the medfly population in Morocco include, in addition to mass trapping and chemical control, the development of new biopesticides, biological control, or the use of a self-limiting genetic approach. Further, setting up an integrated long-term ecological monitoring program and incorporating new technologies into monitoring programs is an urgent need to fight this devastating pest. This could be facilitated by natural resources that can support growth in this sector. Indeed, the African regions have the most incredible plant diversity on the planet. Genetic modifications of plants and medflies could be done to improve the molecular mechanisms that control the interplay between plants and medflies. An advanced understanding of the medflies–plant interaction will be an effective tool for protection. Finally, the current trend is to combine all these methods in an integrated *C. capitata* management strategy.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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