



The biological activities of *Citrus* species in crop protection

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ABSTRACT

Citrus, a globally cultivated genus, is rich in bioactive compounds like flavonoids and essential oils, known for their health benefits and antimicrobial properties. This review explores the potential of *Citrus* extracts, essential oils, and bioactive compounds in enhancing crop protection. Integrating these natural products into pest management strategies has the potential to reduce reliance on chemicals, address resistance issues and promote sustainable agriculture and rural economies. Chemicals have been widely used for crop protection, providing immediate benefits but also posing significant risks to human health and the environment, such as water and air pollution, soil degradation and the development of resistant pest strains. This comprehensive review highlights the importance of citrus-based solutions in modern agriculture, which aims to balance productivity with environmental and health concerns. It also summarises the broad spectrum of activity of these fruits against a wide range of plant micro-organisms (insects and mites, bacteria, fungi, viruses, nematodes) and weeds. As research continues to explore their full potential, *Citrus* waste is poised to play a crucial role in advancing sustainable and resilient agricultural practices.

1. Introduction

Citrus L. (Rutaceae), native to the subtropical and tropical areas of Asia [1], includes several species of economic interest such as *Citrus x limon* (L.) Osbeck, *Citrus x aurantium* L. – bitter orange, *Citrus x sinensis* (L.) Osbeck – sweet orange, *Citrus x reticulata* Blanco – mandarin, *Citrus x paradisi* Macfad – grapefruit, *Citrus x bergamia* (Risso) Risso & Poit. – bergamot orange, *Citrus x medica* L. – citron, and many others [2]. Due to the frequent formation of hybrids, and the introduction of several cultivars through cross-pollination, there is a continuous reformulation of *Citrus* taxonomy and phylogeny [3–5]. Citrus is one of the most important crops, cultivated worldwide with an annual global production of about 150 million tons [6]. The world production of citrus has increased almost 5.5 times in the last 60 years and the major citrus producing countries include China, Brazil and India [7]. Citrus fruits are grown in more than 140 countries across the world, being popular among consumers due to their characteristic flavours and nutritional value, but also because these are an important source of bioactive

compounds, mainly phenolic compounds (flavonoids, phenolic acids, and coumarins), terpenoids (limonoids and carotenoids), and pectin [8]. Among them, flavanones and many poly-methoxylated flavones are synthesized by the plant itself and they have a key role in the control of responses against pathogens [9]. Various studies, primarily focusing on flavonoids and essential oils (EOs), have demonstrated the beneficial activities of citrus compounds including antioxidant, antibacterial, antidiabetic, antifungal, neuroprotective, and anti-inflammatory activities, as well as antitumor potential [10,11]. The health benefits of citrus fruits are mainly due to their abundance of nutrients and bioactive compounds, including carbohydrates, proteins, lipids, vitamins, and minerals [12], which contribute to their health-promoting properties, and make them an excellent addition to a balanced diet [13]. For these reasons, they have potential applications in various industries such as pharmaceuticals, cosmetics and food. Citrus EOs are effective in crop protection and offer a sustainable and environmentally friendly alternative to synthetic agrochemicals [14]. Synthetic agrochemicals such as fungicides, insecticides, acaricides, herbicides, algicides, bactericides,

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fumigants, termiticides, repellents, molluscicides, nematicides, and pheromones have long been the most widely used method of crop protection [15]. While agrochemicals are commonly used for their immediate effects, their excessive and inappropriate use poses significant risks to human health and the environment, including water and air pollution, soil degradation, the death of non-target organisms, the emergence of resistant strains, loss of biodiversity and health hazards [16,17]. Moreover, considering the increase of the regulatory limitations on the use of synthetic agrochemicals and the growing issue over chemical residues in agricultural products, there is an urgent necessity to develop and adopt biological control strategies for pest management [18]. In this context, Integrated Pest Management (IPM), as defined by DIRECTIVE 2009/128/EC [19] is based on a careful assessment of all available methods for plant protection and the integration of the most appropriate measures to prevent or contain the development of harmful organisms, minimising risks to human health and the environment. Today, the Future Integrated Pest Management is based on the European Green Deal - Farm to Fork Strategy, which identifies four strategic objectives: (1) increase IPM; (2) reduce pesticide candidates for substitution; (3) increase low-risk pesticides; (4) switch to organic farming [20]. Therefore, in modern agriculture, developing new, efficient and environmentally friendly ways to promote plant growth and crop protection, has become essential. Various strategies such as appropriate crop rotation, reduced use of chemical control methods, and the implementation of organic or integrated farming can be effective [21,22].

Resistance development is particularly problematic, requiring time-consuming and costly solutions [23]. Pathogenic micro-organisms, environmental stresses, and chemical treatments are very common elements in the life cycle of plants. For these reasons, during evolution, plants have evolved different strategies to cope with these threats [24]. The goal is to find new management strategies to prevent multiple resistance and avoid future pest challenges. One promising approach is the use of natural compounds or extracts as potential agrochemicals. These compounds have broad antimicrobial activity [25]. In addition, substances that activate plant resistance can enhance the production of bioactive compounds as natural defences, contributing to more sustainable practices by reducing reliance on agrochemical controls [26]. Every year, 120 million tonnes of waste, mainly peel, membranes and seeds, are produced by the citrus processing industry [27]. The possibility of converting this waste into commercially viable products is urgently needed. These wastes are being explored for innovative uses in the pharmaceutical and biomedical sectors and in green extraction processes for the production of biosorbents, nanomaterials and organic fertilizers [28]. The main objective of this review was to provide a comprehensive overview of the potential use of citrus EOs, extracts, and bioactive compounds for crop protection, highlighting the broad-spectrum activity of these fruits against insects, mites, bacteria, fungi, viruses, nematodes and weeds. The potential use of citrus bioactives, extracts and EOs waste, which can contribute to sustainable land management and a greener agricultural future, was also highlighted.

1.1. The main citrus species of agricultural interest

Classification of *Citrus* genus is particularly complex due to several factors. One of the most important is the high rate of hybridization between species. This has resulted in a wide range of fertile hybrids and intermediates, making it difficult to draw clear boundaries between species. In addition, the long history of human breeding has contributed to this complexity: humans have selected and propagated plants with desirable traits, creating a large number of cultivars that, although genetically similar, can have significant differences [29]. Added to this, the phenotypic plasticity of *Citrus* plants played a key role in driving morphological variation in response to different environmental conditions. The combination of these factors has created a genetic and morphological mosaic that challenges conventional taxonomic definitions and makes *Citrus* classification a constantly evolving field [30].

At the beginning, studies of relationships between genera and species were mainly based on morphological characteristics. However, this classification was limited and did not take into account the complexity of *Citrus* genetics. Later, several classification systems were developed, until phylogenetic analyses suggested that there were only three true species within cultivated citrus: citron (*C. medica*), mandarin (*C. reticulata*), and pomelo (*C. maxima*) [31]. Other genotypes were found to be hybrids of these true species. Recent biochemical and molecular marker studies have supported this concept [32].

Citron (*C. medica*) is a large fragrant fruit with a bumpy rind and thick white albedo that is barely edible. The pulp is usually less juicy than other citrus fruits and has a variable flavour, ranging from sour to sweet. As the epithet of its scientific name suggests, it has medicinal properties and was once considered an antidote to poison [33]. All parts of *C. medica* contain numerous bioactive compounds, that contribute to a wide range of health benefits. These include anthelmintic, antiulcer, estrogenic, cardioprotective, and anti-diabetic properties [34]. Beyond its health benefits, citron is a versatile and valuable ingredient with a wide range of applications. In cosmetics, citron-derived EOs are used in perfumes, lotions, soaps and other personal care products for their aromatic and antibacterial properties [35].

Pomelo (*C. maxima*), also known as *C. grandis*, is the largest of the citrus fruits, with a diameter that can vary from 15 to 25 cm and a weight that can reach 2–3 kg. The peel is thick and spongy, varying from green to yellow, while the internal pulp can be white, pink or red, depending on the variety. The flavour of the pomelo is sweet with a slight bitter note [36]. Various parts of the fruit have unique characteristics that can be applied to different industries. The pulp of the fruit is prized for its high levels of vitamins, minerals and antioxidants. This makes it not only a nutritious food, but also a useful ingredient in treatments for digestive disorders and to improve heart and metabolic health [37]. The peel, on the other hand, is rich in EOs, flavonoids and terpenoids, which are used both in medicine and in the food and cosmetics industries, where the EOs are used to flavour and perfume various products, while the pectin extracted from the peel is used as a food additive [38,39]. The seeds of *C. maxima* contain limonoids and flavonoids with natural antioxidant, antibacterial and herbicidal activity [40]. Pomelo leaves and flowers also offer significant benefits. They are rich in volatile compounds and flavonoids and have sedative, anti-epileptic and antibacterial properties. These extracts are traditionally used to treat fever, epilepsy and gastrointestinal disorders, while the flowers, with their aromatic EOs are valuable in the production of perfumes and cosmetics [41].

Mandarin (*C. reticulata*) is a historical citrus fruit with numerous varieties and hybrids. These include well-known varieties such as the clementines (*C. reticulata* var. *clementina*), the mandarin (*C. reticulata* var. *tangerina*), and the Mediterranean mandarin (*C. reticulata* var. *deliciosa*) [42]. Native to the subtropical and tropical regions of Asia, particularly China, mandarins are now widely cultivated in warm temperate and tropical regions worldwide [43]. The mandarin fruit, like other oranges, is smaller, more flattened, round, orange in colour, sweet in taste, with a thin, loose, easily peelable skin, and is easily damaged by cold. It is more popular around the world because its flavour is less acidic, sweeter, and stronger than that of orange [44]. Mandarins account for 22–25 % of global citrus production among commercially cultivated citrus species [45]. Mandarins are valued for their many numerous biological properties, including antioxidant, anticancer, anti-inflammatory, anti-hyperlipidaemic, and anti-diabetic effects [46]. Common fruit's phytochemicals are organic acids, sugars, and amino acids, as well as carotenoids, polyphenols, flavonoids, phenolic acids, and limonoids [47]. Alongside these three founding species, which form the genetic basis of many modern citrus, there are other *Citrus* species that play a central role in global agriculture.

The sweet orange (*C. sinensis*) is one of the largest known citrus cultivar groups in the world, accounting for approximately 70 % of the total annual production of citrus species [11]. The largest producer of

C. sinensis is Brazil with an average annual production of 18 million tonnes, while the USA is second with 8 million tonnes per year [29]. Oranges are consumed worldwide as an excellent source of vitamin C, a powerful natural antioxidant that boosts immunity. Oranges are also rich in secondary metabolites that contribute to the pharmacological activity of the plant [48]. Traditionally, they have been used to treat conditions including constipation, spasm, colic, dysentery, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorders, angina, hypertension, anxiety, depression, and stress [49].

Bitter orange (*C. aurantium*) is a very versatile plant, that produces medium-sized fruits, with thick and wrinkled skin that can vary in colour from green to yellow-orange. Unlike the sweet orange (*C. sinensis*), the pulp of the bitter orange is more acidic and bitter, making it less popular for fresh consumption but ideal for other uses [50].

Lemon (*C. limon*) is an evergreen tree with yellow, oval, elongated, and pointed edible fruits. The exact origin of its natural habitat is uncertain, but it is believed to be native to either Northwestern or North-eastern India [3]. The primary raw materials of *C. limon* are its fruits, particularly the EOs and juice extracted from them. Recent scientific publications have increasingly focused on the diverse pharmacological activities of *C. limon* fruit EOs [51], including their antibacterial, antifungal, anti-inflammatory, and anticancer properties [52]. The rich chemical composition of *C. limon* underpins its pharmacological potential. The fruit contains a significant group of secondary metabolites, such as flavonoids, phenolic acids, coumarins, carboxylic acids, amino acids, and vitamins. The main components of its EOs are monoterpenoids, particularly D-limonene. These valuable chemical constituents contribute to the prominent role of *C. limon* in the food and cosmetic industries [53].

Lime (*C. aurantiifolia*) is a fruit that is gaining popularity among citrus growers around the world due to its adaptability to different agro-climatic and soil conditions, low input costs, year-round fruit-bearing, excellent fruit shelf-life, and steady domestic market demand [54]. Lime fruits are ellipsoidal, measuring 2.5–5 cm in diameter, initially green and yellow when ripe. The greenish-yellow flesh yields a tart, aromatic juice and contains a few white seeds [55]. The EO from the rind is widely used in the pharmaceutical and cosmetic industries for products such as medicines, perfumes, soaps, body lotions, detergents, and food or drink flavouring [56]. There are documented cases of adverse reactions to lime, mainly skin-related problems such as dermatitis or phytophotodermatitis from contact with the peel or juice [57]. Lime contains coumarins, carotenoids, alkaloids, and other compounds with numerous pharmacological properties [58]. Traditional uses and phytochemical properties of *C. aurantiifolia* include antibacterial, antidiabetic, antifungal, antihypertensive, anti-inflammatory, antilipidemic, antioxidant, antiparasitic, and antiplatelet activities. It is used to treat cardiovascular disease, liver disease, osteoporosis, and urolithiasis diseases and acts as a fertility enhancer and insecticide [59].

Grapefruit (*C. paradisi*) is an important *Citrus* species native to Barbados. China, US and Mexico are now major producers [60]. It is consumed fresh or processed into juice, which is commonly used in fruit cups, salads, gelatines, puddings, and tarts. Grapefruit is also processed into jam and jelly. The juice is marketed fresh, canned, dehydrated as powder, or concentrated and frozen [61]. Grapefruit can be made into vinegar or carefully fermented into wine, and naringin extracted from the inner peel is used as a bittering agent in tonic drinks, bitter chocolate, ice cream, and ices. Recent studies have shown that extracts of citrus peels and juices, and their biologically active constituents, have numerous beneficial effects, including antioxidant, antimicrobial, cardiovascular, anticancer, and antidiabetic activities [62].

2. Materials and methods

2.1. Search strategy

A systematic review of *Citrus* EOs, extracts and bioactive compounds reported in literature and their biological activities in crop protection against bacteria, fungi, insects and mites, viruses, nematodes, weeds, and viruses, was performed, covering from March 13, 2024 to March 8, 2025. The search was conducted in English using PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) and Scopus (<http://www.scopus.com>) with different keywords, including “*Citrus* species, *Citrus* fruits, organic agriculture, crop protection, integrated pest management, plant-based agrochemicals, *Citrus* essential oils, *Citrus* bioactive compounds, extraction of *Citrus* functional compounds, antimicrobial activity, antibacterial activity; antifungal activity; nematocidal activity; insecticidal activity; herbicidal activity, antiviral activity, phytopathogens, plant protection, *Citrus* waste”.

2.2. Study selection

The articles cited in this review were selected from reputable journals, based on their scientific relevance and data reliability. A total of 13 *Citrus* species were considered (Table S1, Supplementary Material). For a better understanding of the studies presented in this review, the tables proposed in the Supplementary Materials have been prepared, showing the main results related to the citrus species considered in terms of antibacterial, antifungal, nematocidal, insecticidal and acaricidal, herbicidal, and antiviral activity.

3. Citrus biological activities against plant pathogens

Plant pathogens and pests represent a major challenge in agriculture, particularly due to the limited availability of products with favourable toxicological profiles (classified as low-risk pesticides by REGULATION (EC) No 1107/2009 [63]). The use of agrochemicals is restricted in the European Union because of environmental concerns, as since they can have adverse effects on the environment, and on human and animal health (REGULATION (EC) No 1107/2009; REGULATION (EC) No 396/2005 [63,64]) [65,66]. There is also a risk of the development of resistance in pathogen populations. This is one of the main reasons for the strict regulation of antibiotic use as resistance can potentially be transferred to bacteria that affect humans and animals [67]. For these reasons, new approaches (Sustainable use of pesticides: DIRECTIVE 2009/128/EC [68]) are now gaining ground, such as the combination of copper compounds with EOs or plant extracts (which can increase their efficacy) or the use of bioactive natural products for plant protection and sustainability, or the use of systemic resistance inducers, that can enhance the natural plant defence against bacterial infection [69]. On a chemical basis, many plant metabolites such as flavones, flavonoids, quinines, tannins, terpenes, and saponins isolated from neem, citrus, garlic, *Swinglea glutinosa* (Blanco) Merr., and moringa, have been used for the treatment of various bacterial and fungal diseases. Some phytochemicals appeared to be effective substances against harmful microorganisms and pathogens. EOs have been increasingly recognized for their potential in plant protection due to their natural origin and diverse biological activities. Among the various EOs, those derived from *Citrus* species have the broadest range of applicability [51,70].

Citrus EOs are effective against several fungal pathogens, including those that cause powdery mildew and root rot. These biological properties are due to the strong synergistic activity of the active compounds present in the oils and they can be useful in preventing infections and in protecting plants from disease, to promote healthier growth [71]. For instance, the REGULATION (EU) No 2019/1009/EC [72] classifies citrus EOs in the product function category “PFC 6: Plant biostimulant”. Plant biostimulants are products (substances and/or micro-organisms) whose function is to stimulate plant nutritional processes, regardless of the

nutrient content of the product. Biostimulants have no direct effects on pests or pathogens and therefore do not fall into the category of plant protection products. The aim is to improve the crop quality by increasing nutrient availability and the tolerance to abiotic stress. The European market for biostimulants is growing rapidly and citrus EOs can also be used for the purposes indicated in the above-mentioned European Union Regulation [72].

Citrus EOs can be applied by different methods depending on the type of pests or disease: (i) on the plant surface by spraying EOs on the leaves and stems of the plants; (ii) in the soil to control soil-borne pathogens, pests, and weeds, protecting the plant roots and improving overall plant health or (iii) by treating seeds with EOs before planting. Unlike synthetic products, citrus EOs are natural and biodegradable, making them environmentally friendly [73]. They are also recognized as safe for use in agricultural practices due to their low toxicity to humans, animals and beneficial insects such as bees. The use of EOs can help in resistance management. However, high concentrations can cause phytotoxic effects, such as leaf burn or stunted growth. In addition, EOs are volatile and can degrade when exposed to light and air. Formulation studies are essential to improve their stability and efficacy. Today, citrus EOs are widely available commercially and are used in various industries, including agriculture, personal care, and aromatherapy [74, 75]. Citri-V™ is a vaporized blend of citrus EOs (orange: bergamot, 1:1 v/v) that is effective against *Penicillium chrysogenum*, *Aspergillus niger*, and *Alternaria alternata* [76]. Prev-Am®, (Oro Agri, Cape Town, South Africa) contains 5–6 % orange oil as the active ingredient, with D-limonene as the main component [77]. At least 7 formulations are currently available in Italy, including a high concentration (67 % w/w) formulation manufactured by ARYSTA LifeScience Benelux SPRL and distributed by UPL Italia Srl. Ongoing research is focused on optimising the formulations and delivery methods of citrus EOs to maximise their protective effects while minimising any adverse effects on plants. Studies are also exploring the synergistic effects of combining citrus EOs with other agrochemicals for enhanced crop protection [78]. Recently, the European Food Safety Authority (EFSA) was asked by the European Commission to provide scientific support for the approval of lemon EOs for use in plant protection as acaricides, insecticides, and fungicides on fruit trees [79]. With a variety of commercial products available, these oils can be easily integrated into agricultural practices to improve plant health and yield.

3.1. Antibacterial activity

A review of the literature showed that EOs from *C. aurantium*, *C. limon*, *C. aurantifolia*, *C. reticulata*, *C. sinensis* and *C. paradisi* have been tested for their antibacterial activity against several phytopathogens. Citrus EOs can inhibit the growth of Gram-negative and Gram-positive bacteria, including some phytopathogenic strains. The antibacterial activity was evaluated by measuring either the Minimum Inhibitory Concentration (MIC), the Minimum Bactericidal Concentration (MBC), inhibition zones, or percentage inhibition (Table S2, Supplementary Material).

C. aurantium var. *amara* was evaluated for its bactericidal activity against several pathogens, including *Erwinia amylovora*, *Pseudomonas savastanoi* spp. *Savastanoi*, *Xanthomonas vesicatoria*, and *Allorhizobium vitis*. Although these substances have shown significant efficacy *in vitro*, *in vivo* assays have not shown significant reductions in disease incidence or changes in population dynamics, probably due to problems with emulsion stability. Therefore, although EOs and hydrolates have potential as agricultural defence tools, optimization of their formulation (e. g., through nanoemulsions, and microencapsulation) is essential to improve their biological activity and stability over time [80]. *C. aurantium* leaves and small branches EOs showed potential antibacterial activity against *Agrobacterium tumefaciens*, *Dickeya solani*, and *E. amylovora*. Better inhibition zone values (mm) were obtained by branch EOs against *A. tumefaciens*, while leaf/twig EOs showed higher

activity against *D. solani* and *E. amylovora* [81].

Xanthomonas campestris pv. *citri* is responsible for many plant diseases, such as leaf blight, black rot, bacterial leaf spot, and citrus canker, which cause crop yield losses in economically important crops worldwide [82]. *C. reticulata*, *C. sinensis*, *C. aurantium* and *C. limon* reported strong antimicrobial activity against ten strains of Gram-negative and Gram-positive bacteria, including several phytopathogenic strains such as *X. campestris* pv. *citri*. Specifically, *C. limon* showed the lowest MIC values of 15 µg/mL for *X. citri* strains NCPPB 3236 and NCPPB 3562, and 20 µg/mL against *X. citri* strain NCPPB 3832 [83].

Erwinia herbicola (syn. *Pantoea agglomerans*) is widely distributed in agricultural and natural environments. *C. aurantium* EO can inhibit the growth of *E. herbicola*, which causes several post-harvest diseases in fruits and vegetables [84].

Mirzaei-Najafgholi et al. [85] evaluated the effects of EOs from *C. aurantium* and *C. aurantifolia*, and their major constituents, including citral, linalool, citronellal, geraniol, α-terpineol, and linalyl acetate, which showed antibacterial activity against *Xanthomonas citri* pv. *citri* (Xcc). Xcc is widespread, has a broad host range and it is responsible for the most severe canker symptoms. Notably, *C. aurantifolia* EO and citral showed the highest antibacterial activity. Synergistic effects were observed between α-terpineol – citral, citral – citronellal, citral – geraniol, and citronellal – geraniol combinations. Transmission electron microscopy revealed that citral exposure caused cell wall damage and altered cytoplasmic density in Xcc cells [85].

Clavibacter michiganensis subsp. *Sepedonicus* (Cms) is a major cause of potato ring rot and *Clavibacter michiganensis* subsp. *insidiosus* (Cmi) is responsible for bacterial wilt of alfalfa. *C. aurantifolia* EOs were effective against both bacteria, while *C. limon* showed effectiveness specifically against Cms [86]. The mode of action of EOs against microorganisms involves several mechanisms [87]. These include the attack on the microbial cell wall, which leads to an increase in permeability and loss of cellular constituents. This results in the acidification of the cytoplasmic contents, blockage of cellular energy production and synthesis of structural components, and ultimately destruction of genetic material, leading to microbial death [88]. The synergistic activity of combined EOs may be attributed to their effects on the microbial cell membrane. The antimicrobial efficacy of citrus EOs is often enhanced by the interactions between their constituents. When tested in synergy, they show greater potency than when their major components are tested individually [89]. In this context, *C. aurantifolia*, *C. paradisi* and *C. limon* EOs showed better activity when tested in combination with microorganisms associated with fruit spoilage (*Serratia marcescens* and *Erwinia cacticida*) [90]. Recently, *C. aurantifolia*, has been confirmed to possess a very strong inhibitory activity against *S. marcescens* [91].

From an agricultural point of view, all these results provide a further confirmation that Citrus EOs may be used as a potential natural antimicrobial agent in the food industry.

3.2. Antifungal activity

Many citrus EOs have been reported in the literature for their antifungal activity (Table S3, Supplementary Materials). A variety of methods were used in the studies, such as agar diffusion, broth microdilution, food poisoning techniques, volatile phase assays, disc diffusion, and microdilution broth methods. Activity was evaluated by measuring either the MIC, Minimum Fungicidal Concentration (MFC), inhibition zones, or percentage inhibition.

C. sinensis EO has been evaluated for its antifungal properties against a wide range of fungal species. The study by Sharma et al. [92] provides comparable results of *C. sinensis* EO tested against *Aspergillus niger*, *Lasiodiplodia theobromae*, *Alternaria alternata*, *Cladosporium fulvum*, *Botrytis cinerea*, *Penicillium expansum*, *Alternaria chartarum*, *Alternaria mali*, *Penicillium chrysogenum*, *Cladosporium cladosporioides*, and *Parasitocium roridum*. The poisoned food technique and the volatile activity assay showed 100.0 % inhibition at 700 ppm for most of them

[92]. EO extracted from the peel of *C. sinensis* inhibited the fungal growth of *P. chrysogenum*, with an inhibition zone of 18.99 mm, and a MIC of 9.33 $\mu\text{L}/\text{mL}$ [93]. Elgat et al. used the radial growth technique method and observed significant inhibition of *Aspergillus flavus* ($86.66 \pm 0.33\%$), *A. niger* ($96.00 \pm 4.00\%$), and complete inhibition of both *Aspergillus terreus* and *Fusarium culmorum* [94]. In line with these findings, Velázquez-Núñez et al. [95] confirmed the antifungal activity of orange peel EO against *A. flavus* by comparing the effectiveness of two application methods: direct contact and vaporization. The MIC value for direct contact was found to be 16000 mg/L, while for the vaporization method it was significantly lower at 8000 mg/L of air. This finding suggests that vaporization reduces the amount of oil required and provides more effective control of fungal growth than direct application. Furthermore, the effect of the EO in the vaporization phase was more consistent even at low concentrations, highlighting a greater penetration and inhibitory capacity compared to direct contact [95]. In parallel, Sharma and Tripathi [96] investigated the effect of *C. sinensis* EO on the growth of *A. niger*. EO completely inhibited fungal growth on agar at a concentration of 3.0 mg/mL, which is considered fungicidal. At lower concentrations, such as 1.5 mg/mL, a fungistatic effect was observed, with a 79 % reduction in growth after 7 days and a delay in conidia formation [96]. In more recent studies conducted by Samandari et al. [97], *A. alternata* had an inhibitory concentration (IC_{50}) value of $326 \pm 20 \mu\text{g}/\text{mL}$, with MIC and MFC values of $700 \pm 59 \mu\text{g}/\text{mL}$ and $1000 \pm 59 \mu\text{g}/\text{mL}$, respectively, with 100 % inhibition achieved at 800 $\mu\text{g}/\text{mL}$. *Alternaria dumosa* had an IC_{50} of $176 \pm 2 \mu\text{g}/\text{mL}$, with MIC and MFC values of $550 \pm 29 \mu\text{g}/\text{mL}$ and $900 \pm 59 \mu\text{g}/\text{mL}$, respectively, with complete inhibition at 600 $\mu\text{g}/\text{mL}$. *Alternaria atra* had an IC_{50} of $360 \pm 12 \mu\text{g}/\text{mL}$, an MIC of $750 \pm 29 \mu\text{g}/\text{mL}$, and an MFC of $1000 \pm 59 \mu\text{g}/\text{mL}$, with 100 % inhibition observed at 800 $\mu\text{g}/\text{mL}$. These results suggest that *C. sinensis* EO has significant antifungal properties, exhibiting both inhibitory and fungicidal activity against various fungal pathogens, despite the different assays used. Its efficacy varies depending on the fungal species and shows promise as a natural antifungal agent for the treatment of fungal infections.

The antifungal properties of *C. limon* EO have been extensively studied, demonstrating its efficacy against several fungal species. Mbili et al. reported complete inhibition of *B. cinerea* at a 20 % concentration in the volatile phase assay, with 52 % inhibition at a 1 % concentration [98]. Studies by Simas et al. showed that *C. limon* EO had a MIC of 312 $\mu\text{g}/\text{mL}$ against *B. cinerea* [99]. *In vitro* studies have demonstrated the antifungal activity of *C. limon* EO against *B. cinerea*. The study by Vitoratos et al. [100] extended this knowledge by demonstrating that such effects result in an effective reduction of disease severity in inoculated fruits *in vivo*. Lemon EO at concentrations of 0.05 $\mu\text{L}/\text{mL}$ completely inhibited the growth of the pathogen on strawberries and significantly reduced the infection in other fruits, such as cucumbers. Treated fruits showed a significant delay in the onset of symptoms compared to control samples, where the infection was visible within 48 h. Furthermore, the EO demonstrated the ability to inhibit the germination of *B. cinerea* spores under *in vitro* conditions, confirming its potential as an antifungal agent [100]. With regard to fungicidal activity, agar well diffusion studies reported MFC of 100 mg/mL for *A. niger*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, and *Rhizopus oryzae*. *A. flavus*, *Fusarium avenaceum* and *Fusarium solani* required MFCs above 100 mg/mL for complete inhibition. *A. alternata* had a much lower MFC of 50 mg/mL, and both *P. digitatum* and *Penicillium expansum* were effectively inhibited at a MFC of 25 mg/mL. Other fungi such as *Rhizopus stolonifer*, *Geotrichum candidum*, *Mucor piriformis*, and *Mucor racemosus* showed MFCs ranging from 50 mg/mL to 100 mg/mL [90]. The antifungal activity of lemon EO against *A. niger*, *A. flavus* and *Rhizopus* spp. was also tested by Souza et al. [101]. *Eutypa* sp., *Botryosphaeria dothidea*, and *Fomitiporia mediterranea* are important pathogens that attack vine wood and cause serious losses in terms of production and quality in vineyards. Significant growth inhibition of these fungi was demonstrated by Ammad et al. [102]. The minimum concentration of 0.25 %

produced an inhibition of 82 % on *Eutypa* sp., 48 % on *B. dothidea* and 33 % on *F. mediterranea*. In continuation of the studies on the antifungal activity of *C. limon* EO, Dimic et al. extended the field of study by evaluating the biological activity in both direct contact and vapor phase, applied against food spoilage molds, including *A. parasiticus*, *C. cladosporioides*, *Eurotium herbariorum*, *P. chrysogenum* and *Aspergillus carbonarius*. However, some significant differences were observed in the responses of different pathogens depending on the concentration used [103]. In conclusion, *C. limon* EO was particularly effective against common postharvest pathogens like *Aspergillus*, *Penicillium*, and *Botrytis*, making it a promising candidate for natural antifungal agents in the agricultural and food industries.

C. aurantifolia EOs exhibit antimicrobial activity against a wide range of microorganisms, with varying degrees of efficacy depending on the method and microorganism tested. In 2013, a research study demonstrated the activity against *Mucor hiemalis*, *P. expansum* and *F. proliferatum* using the agar dilution method with varying degrees of efficacy [104]. Studies by Rammance et al. showed that the oil was effective against *A. flavus* and *A. parasiticus*, with minimum inhibitory concentrations (MICs) of 1 % and 4 % (v/v) using the agar diffusion and broth microdilution method [71]. In addition, *P. expansum* was inhibited by 52 % at a concentration of 2000 ppm, while *F. proliferatum* showed a stronger inhibition of 91.5 % at the same concentration. The MIC values for *Colletotrichum gloeosporioides* and *R. stolonifer* were reported to be 0.5 % (v/v). More recently, using a volatile phase assay, Mbili et al. showed that the oil inhibited *B. cinerea* by 63 % at 1 % [98]. *B. cinerea*, tested by Simas et al. [99] reported a MIC of 625 $\mu\text{g}/\text{mL}$. Ajayi-Moses et al. [90] tested the EO against several fungal species using the agar well diffusion method. The MFCs for *A. niger*, *A. fumigatus*, *A. parasiticus*, *R. oryzae*, and *R. stolonifer* were reported to be 100 mg/mL. The MFC for *G. candidum* was 50 mg/mL, while *A. alternata* required 75 mg/mL. Some fungi, such as *F. avenaceum* and *F. solani*, required concentrations above 100 mg/mL for fungicidal activity. Notably, *P. digitatum* and *P. expansum* were highly susceptible, with MFCs of 25 mg/mL, and *M. piriformis* and *M. racemosus* had MFCs of 50 mg/mL. Previous studies have focused on the antifungal activity of EOs obtained by hydro-distillation from citrus peels, showing remarkable efficacy against pathogens. However, the activity of EOs obtained from *C. aurantifolia* leaves against *Phaeoramularia angolensis* was also evaluated. The oils showed significant antifungal activity *in vitro*, with MICs ranging from 1.4 to 1.5 mg/mL. Effects observed include complete inhibition of the fungus at these concentrations, preventing regrowth even after prolonged exposure [105]. Although *in vitro* studies have demonstrated the potential of lime EOs, their efficacy under *in vivo* conditions requires further investigation. The study by Bosquez-Molina et al. [106] demonstrated the efficacy of lime EO in controlling fungal infections caused by *C. gloeosporioides* and *R. stolonifer* in papaya fruit. In particular, the preventive treatment gave the best results: in the case of *C. gloeosporioides*, the concentration of 0.14 % (w/w) completely inhibited the development of the disease, while in the case of *R. stolonifer*, the application resulted in a 40 % reduction in infection, compared to untreated controls. When the treatment was applied after the infection, the efficacy was lower, suggesting that the preventive action of the EO was more effective than the curative action [106].

The antifungal activity of *C. aurantium* flowers EO was tested against a panel of fungal strains including *Aspergillus* spp., *Fusarium* spp. and *A. alternata*, with results indicating significant inhibition of mycelial growth. *C. aurantium* EO showed strong activity against *F. graminearum* and *A. flavus*, with an inhibition zone diameter of 22.0 mm, and MFC values ranging from 0.08 mg/mL to 1.25 mg/mL, depending on the fungal species. *F. oxysporum* and *A. niger* were moderately susceptible, while *A. alternata* was less susceptible with a higher MFC value. The antifungal activity of the oil has been attributed to the synergistic action of its main constituents, particularly limonene and E-nerolidol, which interfere with the integrity of the fungal cell membrane and compromise its vitality [107].

C. reticulata was tested against various plant pathogens, highlighting its efficacy compared to other *Citrus* EOs. MIC values showed that *A. alternata*, *Rhizoctonia solani* and *Curvularia lunata* were completely inhibited at 0.2 mL/100 mL, while *Fusarium oxysporum* and *Helminthosporium oryzae* showed greater resistance, with a MIC above 0.2 ml/100 ml. Furthermore, fungal sporulation was completely inhibited at 2 ml/100 ml for almost all pathogens tested [108].

Few studies have focused on comparing and testing different citrus species in parallel. Kuate et al. [109] analysed the effect of EOs extracted from different varieties of citrus on the radial growth and sporulation of the fungus *P. angolensis*, which causes leaf spot and fruit spot disease in citrus fruits. Among the varieties analysed, the differences in antifungal activity were significant and correlated with the degree of tolerance of the varieties to the pathogen. EOs from varieties considered tolerant, such as *C. maxima*, showed the greatest efficacy in reducing the radial growth of the fungus after 10 days, with inhibitions exceeding 61.60 % at a dose of 2500 ppm. In contrast, oils from highly susceptible varieties, such as *C. paradisi*, were more effective in reducing sporulation, with a reduction of more than 64 % at the 1000 ppm dose. These differences suggest that the chemical composition of EOs is highly variable between varieties, with an impact on their antifungal efficacy [109]. Some studies also showed significant differences in *in vivo* tests. Three different citrus species (*C. sinensis*, *C. limon*, and *C. bergamia*) were tested against *Phytophthora infestans*, the major cause of potato late blight. Bergamot EO was the most effective, reducing leaf infection rates by up to 69 %. This was followed by orange oil with a reduction of 42 %. Lemon EO was less effective, reducing infection by less than 1 % [110]. The biological activity of various *Citrus* sp. (*C. limon*, *C. reticulata*, *C. paradisi*, and *C. sinensis*) against four species of food-borne molds (*A. niger*, *A. flavus*, *P. chrysogenum* and *Penicillium verrucosum*) was also evaluated. Orange EO was most effective against *A. niger*, showing the greatest reduction in mycelial growth at the lowest concentrations, with complete inhibition at the 0.94 %. Mandarin EO showed the greatest activity against *A. flavus*, significantly reducing mycelial growth already at lower concentrations. Grapefruit was the most effective in reducing the growth of *P. chrysogenum* and *P. verrucosum* [111]. *Rhizopus nigricans*, *A. flavus*, *M. racemosus*, *Penicillium citrinum* and *B. cinerea* were other saprophytic molds of food origin exposed to the activity of *C. limon* and *C. sinensis* EOs. Lemon EO exhibited the highest antifungal activity against *R. nigricans* with an inhibition zone diameter of 14.15 mm, followed by *A. flavus* (13.20 mm) and *M. racemosus* (12.67 mm). Orange EO showed greater overall efficacy, with the largest zone of inhibition observed on *R. nigricans* (17.23 mm), followed by *P. citrinum* (15.59 mm) and *M. racemosus* (13.97 mm). Furthermore, the MIC and the MFC values indicate that orange EO generally required lower concentrations to exert a fungicidal effect compared to lemon oil [112]. Singh et al. mixed EOs from *C. maxima* and *C. sinensis* in a 1:1 ratio and analysed their efficacy against several food-contaminating molds. However, data analysis indicated that the mixture did not show a significant synergistic effect compared to the individual oils [113].

Although in smaller numbers, many citrus extracts (*C. sinensis*, *C. limon*, *C. reticulata*, *C. aurantifolia* and *C. hystrix* DC) showed antifungal activity against several phytopathogenic fungi (Table S4, Supplementary Material). The antifungal properties of *C. limon* extracts have been extensively studied over the years, with research focusing on various plant pathogenic fungi (*Aspergillus* spp., *Colletotrichum* spp., *Fusarium* spp. and others), showing interesting results [114–118]. Recent studies using disc diffusion methods have shown significant antifungal activity. *A. flavus* showed a zone of inhibition of 21.30 ± 0.17 mm, with a MIC of 4.00 ± 0.11 mg/mL. *R. solani* and *A. alternata* showed larger inhibition zones of 26.90 ± 0.12 mm and 23.50 ± 0.12 mm, respectively, with MIC values of 2.50 ± 0.10 mg/mL and 3.00 ± 0.11 mg/mL [119].

The study conducted by Ugwuja et al. analyses the efficacy of *C. aurantifolia* ethyl acetate extract in the management of foliar disease of taro (*Colocasia esculenta* (L.) Schott), caused by *Phytophthora colocasiae*, one of the major plant diseases affecting this crop. Taro plants were

inoculated with the pathogen and treated with plant extracts at different concentrations (10 %, 20 %, 30 %), applied as a foliar spray. The results showed that lime juice at a concentration of 20 % eliminated the incidence of the disease, a result comparable to that obtained with the fungicide Ridomil® [120].

The antifungal properties of *C. sinensis* extracts against *Colletotrichum* spp., *Curvularia* spp., *Aspergillus* spp., and *Penicillium* spp. were investigated over several years [114,121]. In addition, aqueous extracts of *C. sinensis* were tested to evaluate their ability to reduce the incidence of pathogenic fungi in pearl millet seeds. The application of *C. sinensis* aqueous extract significantly reduced the presence of these fungi, demonstrating an effectiveness comparable to that of the chemical fungicide. Furthermore, seeds treated with citrus extract showed higher germination and higher seedling vigor than untreated ones [122].

3.3. Insecticidal and acaricidal activity

The increase in crop pests is a major challenge, resulting in reduced yields and significant financial and time losses. While synthetic insecticides and acaricides are generally preferred because of their rapid action and ease of use, their long-term use has caused numerous environmental problems, negatively affecting ecosystems and human health [123]. In addition, the development of resistance in crop pests has been accelerated by the widespread use of these chemicals over the past century [124,125]. Aphids are a significant problem worldwide among the insects of greatest concern to agriculture. Their rapid reproduction capacity facilitates their spread and makes them particularly difficult to control as they cause extensive damage to crops, feed on plant sap and transmit phytopathogenic viruses [126,127]. Plant extracts and EOs are known to be promising alternatives to chemicals. They offer potential benefits due to their natural resistance to pests and reduced environmental and human health impacts. Several citrus extracts and EOs have been reported to have insecticidal and acaricidal activity. A significant proportion of the studies available today focus on the efficacy of citrus extracts and EOs against insects that attack stored agricultural products, such as grains and nuts. However, research and application of citrus extracts and EOs for the control of field pests is gaining ground.

A literature survey provided an extensive list of citrus species EOs (*C. sinensis*, *C. limon*, *C. paradisi*, *C. aurantifolia*, *C. aurantium*, and *C. reticulata*) active against various insects and mites (Table S5, Supplementary Material). *C. sinensis* is well known for its ability to protect stored grains against insects. According to the study by Oyebo et al. *C. sinensis* EO was tested and evaluated for its efficacy as a pest control agent against the primary storage insect pest of *Callosobruchus maculatus* [128]. *C. sinensis* peel EO showed 100.00 % mortality at the highest concentration in the first hour of exposure in the contact toxicity test at 3 % concentration. This result highlights the potent effect of the oil over the hexane extract in rapidly eliminating this pest, confirming its potential as a natural insecticide. The biological activity of EOs extracted from the peels of *C. aurantifolia*, *C. limon*, *C. limetta* and *C. reticulata* was evaluated against *Callosobruchus chinensis* by contact toxicity, fumigant toxicity and repellency tests. In every test, *C. aurantifolia* seemed to be the most promising EO: in contact toxicity it showed a LC_{50} of 4.02 mg/cm², in fumigant toxicity a LC_{50} of 0.57 mg/L after 24 h, while the repellent activity reached the maximum with 92 % at a dose of 2.5 mg/cm² [129]. *Tribolium castaneum* (the red flour beetle) is a significant global pest of stored products, which thrives in controlled indoor environments and is spread by human activity [130]. Known for its rapid population growth and wide host range, the beetle is particularly problematic in wheat and rice mills, and other processed food storage systems [130]. *C. aurantium* showed efficacy against *T. castaneum* in fumigant and repellency tests, with LD_{50} and LD_{90} values of 64.78 µL/L air and 103.55 µL/L air, and a repellency index of 84 % [131]. Recently, *C. limon* was found to cause 93.3 % mortality in larvae and 16.7 % in adults of *T. castaneum* after 14 days, and 13.3 % mortality in larvae and 72.2 % in adults of *Trogoderma granarium* (the khapra beetle), one of the

most important cereal pests with the ability to survive under different climatic conditions after 7 days in contact toxicity tests [132]. The study of Sagheer et al. [133] evaluated the efficacy of EOs extracted from four *Citrus* species (*C. paradisi*, *C. sinensis*, *C. aurantium* and *C. reticulata*) against *T. granarium*. The experiments were conducted by exposing the insect larvae to grains treated with concentrations of 2%, 4% and 8% of the EOs. The results showed that the EO of *C. aurantium* at the concentration of 8% caused the highest mortality rate (27.30%) [133]. *Sitophilus zeamais* (the maize weevil) is a major threat to cereal conservation in tropical and subtropical countries, where high temperature and humidity favour its rapid development [134]. Several studies evaluated the efficacy of *C. aurantiifolia*, *C. sinensis*, and *C. reticulata* against this pest, revealing differences in insecticidal activity [135–137]. *C. reticulata* peel extract exhibited the highest activity across multiple assays, with LC₅₀ values of 51.29 µL/mL (in contact assay), 1.52 µL/g (in ingestion assay), and 41.92 µL/L air (in fumigant assay) [135]. Nevertheless, all studies confirmed the high effectiveness of *Citrus* EOs against *S. zeamais*. *Sitophilus granarius* has adapted to a completely different environment: this weevil finds ideal conditions in temperate climates, where its biology is closely linked to the stability of the storage environment and the ability to survive even at lower temperatures [138]. EO extracted from peels of *C. aurantium* was tested and *S. granarius* proved to be more resistant than other pests, such as *Cryptolestes ferrugineus* and *Liposcelis bostrychophila*, requiring higher concentrations of EO to achieve a significant mortality rate. A mortality rate of over 80% required a dose of 200 µL/L air. Compared to other species tested, *S. granarius* showed a higher lethality threshold, with LC₅₀ and LC₉₀ values of 103.77 µL/L and 184.11 µL/L, respectively, indicating a lower sensitivity to fumigant toxicity compared to other pests [131]. Another study evaluated the fumigant toxicity and repellent activity of *C. limon* EO against *S. granarius* adults. Biological tests were conducted to assess insect mortality due to exposure to vapors. The results showed a mortality of 92.50% after 72 h at 400 µL/L air [139].

Unlike stored products, crops like tomatoes and grapes are grown under complex field conditions where many factors (e.g., weather, crop growth stage, and natural predators) influence pest dynamics. This makes it more difficult to standardise EOs treatments for such environments compared to the controlled atmosphere of storage facilities. Saad et al. [140] evaluated *C. sinensis*, *C. limon*, *C. paradisi*, and *C. aurantiifolia* against *Spodoptera littoralis*, the cotton leafworm. This pest is widespread in Africa and in the Middle East and it is a major agricultural pest causing severe economic losses due to crop destruction. The fumigant toxicity tests showed that *C. sinensis* and *C. aurantiifolia* had the highest efficacy with LC₅₀ values of 6.88 µL/L and 6.84 µL/L, respectively, while *C. limon* and *C. paradisi* had LC₅₀ values of 15.32 µL/L and 18.01 µL/L. Further research by Ribeiro et al. [141] evaluated *C. limon*, *C. aurantiifolia*, and *C. reticulata* against *Tetranychus urticae*, a cosmopolitan mite that feeds on more than 1,100 documented plant species. *C. reticulata* showed the highest efficacy in fumigant assays with an LC₅₀ of 6.09 µL/L, while *C. limon* showed strong contact toxicity with an LC₅₀ of 25.18 µL/mL. Laarif et al. confirmed the efficacy of *C. aurantiifolia* EO against *S. littoralis* and *Tuta absoluta* with no apparent phytotoxicity on tomato leaves [142]. *T. absoluta* causes severe damage to the tomato industry, especially in protected crops, and is currently considered one of the most devastating invasive pests of solanaceous plants worldwide [143]. Papachristos et al. evaluated the efficacy of *C. sinensis*, *C. limon*, and *C. aurantium* EOs on *Ceratitidis capitata* using dietary exposure assays. *C. capitata*, commonly referred to as the Mediterranean fruit fly, has a remarkable history of destructive infestations, mainly due to the expansion of the international fruit trade [144]. The results highlighted LC₉₀ values of 13.00 µL/g for *C. sinensis* and *C. aurantium*, and 16.50 µL/g for *C. limon*. *C. limon* EO was also shown to cause 80% larval mortality of *Agrotis ipsilon*, a major agricultural pest in tropical and subtropical regions worldwide, causing significant crop losses in countries including Chile, Brazil, Egypt, India, Myanmar, Poland, Spain, and USA [145]. Karamaouna et al. investigated the

contact toxicity of citrus EOs on *Planococcus ficus*, the vine mealybug, which is a serious threat to grape production [146]. The results shed light on the efficacy of *C. sinensis* and *C. limon* against both larval and adult stages of this pest. *C. sinensis* EO showed that the LC₅₀ for larvae was 5.4 mg/mL, while the LC₉₀ was up to 13.5 mg/mL. For adults, the LC₅₀ remained at 5.4 mg/mL, but the LC₉₀ was up to 16.2 mg/mL. In contrast, *C. limon* showed lower LC₅₀ values for both larvae and adults. The LC₅₀ was 3.6 mg/mL, and the LC₉₀ ranged from 14.4 mg/mL to 29.7 mg/mL. For adults, the LC₅₀ was even lower at 2.7 mg/mL, and the LC₉₀ ranged from 14.4 mg/mL to 29.7 mg/mL. These results suggest that *C. limon* may be more effective as a contact insecticide against *P. ficus*. The wider range of concentrations effective in killing the pests with *C. limon* suggests that it may provide more consistent control over varying application concentrations. Zarrad et al. investigated the chemical composition and insecticidal activity of *C. aurantium* EO against *Bemisia tabaci* adults. *B. tabaci*, commonly known as the tobacco whitefly, is a small phytophagous insect considered to be one of the most damaging species to crops worldwide, due to its high reproductive capacity, wide host range, and transmission of phytopathogenic viruses. At the highest concentration (20 µL/L air), 100% mortality of adults was observed within 24 h. *C. aurantium* EO showed an LC₅₀ of 3.97 µL/L air, slightly lower than pure limonene (4.71 µL/L air), indicating a higher efficacy of the EO [147]. The study by Alotaibi et al. analysed the potential use of EOs extracted from the peels of *C. aurantium* and *C. reticulata* for the control of *Aphis punicae* and *Aphis illinoisensis*, harmful parasites of pomegranate and grapevine crops. In laboratory tests, *C. aurantium* EO showed greater efficacy than *C. reticulata* EO, with higher levels of toxicity and a more pronounced repellent effect. Both EOs showed greater activity against *A. punicae* than *A. illinoisensis* [148]. Kayahan analysed the efficacy of *C. limon* and *C. sinensis* EOs in controlling the black bean aphid (*Aphis fabae*). The author evaluated the insecticidal activity of the oils at different concentrations, determining lethality and analyzing their effects on aphid survival and fertility. The results showed a high efficacy of both oils, with *C. limon* showing a superior insecticidal effect compared to *C. sinensis*, significantly reducing the longevity and reproductive capacity of aphids. The results showed that *C. limon* had a more pronounced effect than *C. sinensis*, with LC₅₀ values of 3.86 µL/L for *C. limon* and 4.79 µL/L for *C. sinensis*, while LC₉₀ values were 10.46 µL/L and 11.86 µL/L, respectively. Sub-lethal concentrations (LC₃₀ and LC₄₀) significantly reduced aphid longevity, fertility, and intrinsic growth rate [149]. The aphids *Acyrtosiphon pisum*, *Rhopalosiphum padi*, *A. fabae* and *Macrosiphum euphorbiae* were subjected to fumigation toxicity tests and *in vivo* tests to evaluate the insecticidal activity of *C. aurantium* EO. In fumigation tests, the efficacy of the oil was dose-dependent, with a mortality rate of 98% in *A. pisum*, 74% in *R. padi*, 60% in *A. fabae* and 54% in *M. euphorbiae* at the highest concentration. In *in vivo* tests, faba bean plants infested by *A. fabae* were treated with pure EO and mortality reached 91% after 24 h [150].

Citrus extracts with pest control activity include *C. sinensis*, *C. reticulata*, *C. limon*, *C. aurantium*, and *C. paradisi* extracts have been reported (Table S6, Supplementary Material). *C. maculatus* is the most destructive pest of legumes and cereals, causing 10–20% storage losses [151,152]. In 2016, Oyeboode et al. reported a mortality rate of 99.33 ± 1.15% within the first day when 1% *C. sinensis* peel hexane extract was applied [128]. *C. sinensis* pulverized peel was tested against *S. zeamais* and resulted in a mortality rate of 78.3% [153], demonstrating that not only the essential oils of *C. aurantiifolia*, *C. reticulata* and *C. sinensis* have activity against this pathogen [135–137]. Methanol extracts of the peel and seeds of *C. sinensis* were also tested against *Agonoscyta pistaciae*, one of the main pests of the pistachio tree and showed a low level of activity. The IC₅₀ was found to be 62.04% for peel extract and 43.60% for seed extract. Rouhani et al. also tested the fruit peel methanol extracts of *C. reticulata* where the IC₅₀ was 38.84%, indicating a higher efficacy of *C. reticulata* against *A. pistaciae* compared to *C. sinensis* [154]. In the study by Siskos et al., *C. aurantium* methanol extract achieved 100.0 ± 0.0% mortality at a concentration of 200 µg/cm², indicating a very high

efficacy against *Bactrocera oleae*, which attacks the olive trees [155]. The efficacy of *C. paradisi* and *C. limon* was also evaluated against *C. capitata* and *Anastrepha fraterculus*. Toxicity measured by LC₅₀ showed significantly lower values for *A. fraterculus*, suggesting a higher sensitivity [156]. *A. fraterculus* (the South American fruit fly) attacks a wide range of host plants, including fruits of high commercial value. This species is regulated in international fruit exports and specific treatments are required to ensure compliance. Fruit exporting countries must ensure that products destined for export are free of *A. fraterculus*, often through expensive phytosanitary treatments [157]. The study by Majeed et al. examined the insecticidal efficacy of acetone, water and ethanol extracts of *C. aurantium* and *C. sinensis* against *Drosicha mangiferae*, a serious pest of citrus crops. The tests were conducted on adult female specimens and second-stage nymphs of scale insects. In adults, the most effective extracts were those obtained from *C. sinensis* seeds water extracts, with LC₅₀ values of 29.42 % at 72 h. For the second instar nymphs, which proved to be more sensitive to the botanical extracts than the adults, the most effective compounds were those obtained from the ethanol peel extract of *C. sinensis* and the water seeds extract of *C. aurantium*, with LC₅₀ values of 39.40 % and 51.08 % at 72 h, respectively [158].

All these studies demonstrate that citrus EOs and extracts possess significant insecticidal activity against a wide range of pests. The results for contact and fumigant toxicity are promising. However, the efficacy is highly variable depending on the species of *Citrus* species and the target insect. These results suggest that citrus EO could provide a sustainable alternative to conventional chemical insecticides.

3.4. Nematocidal activity

Plant parasitic nematodes (PPNs) are widespread in nature and can cause significant damage and yield loss to a wide range of agricultural, forestry, ornamental, and medicinal plants. Annually, PPNs are responsible for global losses in the most economically important crops (including vegetables, fruits, and non-edible field crops) [159]. In addition, PPNs are often associated with bacterial and fungal pathogens, that use the nematode's entry pathways into the plant to exacerbate disease symptoms. To manage these complex diseases and improve plant health, it is essential to reduce PPN populations in the soil and suppress soil-borne plant pathogens. [160]. The attack of nematodes on a plant progresses through three stages: (i) initial (asymptomatic), (ii) symptomatic (yellowing, wilting, stunting, yield losses, etc.), and (iii) final stage of parasitism (which can lead to plant death depending on the density of PPN). During each stage, there are changes in the plant as well as in the interactions between the plant, the PPNs, and the biological control agents [161].

The nematocidal activity of *Citrus* species EOs against plant-parasitic nematodes is generally limited. Available studies indicate that *C. sinensis*, *C. aurantium* and *C. reticulata* EOs have shown modest efficacy against species such as *Meloidogyne incognita* and *Xiphinema index*, with lower mortality rates than EOs of other aromatic plants. *In vitro* tests have shown that *Citrus* EOs cause reduced larval mortality and modest inhibition of egg hatching, while soil experiments have confirmed a low capacity to suppress root infestation by *M. incognita*. However, some studies suggest that *Citrus* EOs may have greater efficacy against specific nematode species, such as *Pratylenchus vulnus*, and that its use could be improved through blends with other more active EOs or with advanced formulation strategies to increase its stability and bioavailability [162–164].

Otherwise, several plant extracts have been identified with nematocidal or nematostatic properties. *Meloidogyne* spp. (root-knot nematodes) infest a wide range of important food and economic crops, including various vegetables (e.g., tomatoes, beans, potatoes), field crops (e.g., peanuts, soybeans, sugar cane), ornamentals (e.g., roses, chrysanthemums), and horticultural crops (e.g., passion fruit, kiwi, almonds) [151]. *M. incognita* is distributed in several tropical and subtropical regions of the world, being one of the most destructive pests of

horticultural crops and causing severe losses worldwide [165–167]. Various plant extracts have been reported to be effective against *M. incognita*, usually using egg hatching and mortality of the root-knot nematode [168,169]. Methanol and water extracts of *C. sinensis*, *C. limon*, *C. aurantium* and *C. paradisi* were reported to be effective against *M. incognita* [170–173]. More specifically, Tsai et al. [172] reported that the fresh lemon peel water extract did not affect *M. incognita* second instar larvae after 24 h but paralyzed 90.2 % after 48 h. Fresh orange (*C. sinensis*) and grapefruit (*C. paradisi*) peel water extracts had a mild nematocidal effect and were nematostatic after 24 h, with nematostatic activity increasing to over 80 % after 48 h. After one week of refrigeration, the peel extracts became highly nematocidal, with 82 % of nematodes killed by lemon peel extract after 24 h and 90.8 % after 48 h. Orange and grapefruit peel water extracts showed similar results, with 93.5 % and 85 % mortality, respectively. Fresh orange peel extract slightly inhibited egg hatching after 24 h (33 % after 72 h), while lemon and grapefruit peels had no effect. However, refrigerated peel extracts showed greater inhibition of hatching, with 72.8 % and 76.3 % inhibition for lemon and orange, respectively. Grapefruit peel extract was less effective, with a maximum of 91 % inhibition after 72 h for orange peel extract. Transferring egg masses to distilled water reduced inhibition but still showed significant effects. When applied to soil for two days, refrigerated peel extracts significantly reduced *M. incognita* juvenile infection in mung bean roots, with lemon and orange peel extracts showing similar efficacy, while grapefruit peel was less effective. *M. incognita* can disrupt the normal physiology of plants, leading to poor nutrient uptake, stunted growth, and ultimately a reduction in both the quality and quantity of the crop. In this context, Izuogu et al. suggested that the treatment with 100 % and 75 % aqueous citrus peel extract and 60 g and 40 g of citrus peel powder were comparable to a synthetic nematicide used as a standard [171]. The study by Abolusoro et al. showed that *C. sinensis* peel water extract was effective in causing mortality in *M. incognita* juveniles [170]. Mortality occurred within 6 h for both treatments at all concentration levels. After 24 h, carbofuran caused 100 % mortality at all doses. By day 2, citrus peel water extract also achieved 100 % mortality at all concentrations, except for 25 %, where 96 % mortality was recorded. At 1000 ppm, *C. aurantium* peels and seed extracts can inhibit the hatching of *M. incognita* eggs. Furthermore, a concentration of 1000 ppm of the peel extract resulted in the highest mortality rates [173]. In addition, *C. aurantium* peel and seed methanol extracts were shown to decrease the percentage of hatched eggs of *Rotylenchulus reniformis* and *Tylenchulus semipenetrans* with increasing concentrations. *R. reniformis* is a sedentary root parasite that poses a significant threat to agricultural production in tropical and subtropical regions worldwide [174]. *T. semipenetrans* is the major nematode pest of citrus and is responsible for 'slow decline' disease. It infests citrus, grape, olive, loquat, and persimmon species and cultivars in various greenhouses. The estimated yield loss due to citrus nematodes is 30–50 % [175]. The best mortality test results were obtained with methanol peel extracts against *T. semipenetrans* and seeds methanol extracts against *R. reniformis* [173].

These studies highlighted that citrus extracts can suppress both root and soil population build-up of nematodes and consequently reduce root damage in the tested crops (Table S7, Supplementary Material). The ability of the citrus peel supplement to release plant nutrients and biochemicals that reduce soil nematodes makes them allelopathic plants.

3.5. Herbicidal activity

Weeds are plant species that grow spontaneously in cultivated or managed environments, such as agricultural fields, gardens, lawns, and urban areas. The behavior of weeds is often perceived as problematic because they compete with desired crops or plants for basic resources such as light, water, nutrients, and space. These plants have biological characteristics that make them particularly competitive. Weeds can grow rapidly, produce large amounts of seeds, and are highly resistant to

adverse conditions. Their presence in an agricultural environment can significantly reduce crop yields, interfere with harvesting operations and, in some cases, alter the quality of the final product [176]. Some weeds may also harbor pests or pathogens that are transmitted to the crops [177]. Weed control is a complex challenge. Traditionally, farmers have used manual or mechanical weeding to control weeds, but with the advent of modern agriculture, there has been an increasing reliance on chemical herbicides, which provide more effective control but pose risks to the environment and human health [178]. However, weeds have shown a remarkable ability to develop resistance to many herbicides, making their management even more complicated [179]. For this reason, integrated approaches are being developed that combine agronomic, biological and chemical techniques to minimise the impact of weeds [180].

Most of the available literature on this topic focuses mainly on the use of citrus EOs, rather than extracts, for such applications (Table S8, Supplementary Material). *C. aurantiifolia* EO has been the most studied in terms of inhibition of seed germination and seedling growth on several plants as *Avena fatua* L., *Echinochloa crus-galli* (L.) P. Beauv., and *Phalaris minor* Retz., found in wheat and rice fields [181]. Wild oat (*A. fatua* is one of the most economically damaging weed species in cereal-growing regions worldwide [182]. *A. fatua* is characterized by high seed production, vigorous growth, tall stature, extensive root systems, phenotypic variation, and the ability to germinate under different environmental conditions. It poses a significant economic threat to crop yields due to seed shattering, which allows long-distance dispersal, seed dormancy, which enables seeds to remain viable for several years in the soil, and competitiveness at the seedling stage [183]. These traits make this weed difficult to control and economically maintain.

C. aurantiifolia EO significantly reduced the germination of *A. fatua* and *P. minor* at ≥ 0.25 mg/mL, and of *E. crus-galli* at ≥ 0.50 mg/mL. It also caused a significant reduction in the coleoptile and root growth at ≥ 0.10 mg/mL in *A. fatua* and *P. minor* and at ≥ 0.25 mg/mL on *E. crus-galli* [181]. *C. aurantiifolia* EO is the most preferred due to its moderate toxicity, easy availability, and low-cost extraction process. Therefore, EOs are biodegradable, reducing the environmental chemical burden and mitigating the biomagnification of synthetic chemicals in living organisms. However, further studies are needed to assess the efficacy of *C. aurantiifolia* under field conditions, cost-effectiveness and potential impact on non-target species for commercial use as a natural herbicide [181].

Milk thistle (*Silybum marianum* (L.) Gaertn.) is a broad-leaved weed commonly found in cereal fields. Milk thistle competition can reduce wheat productivity by up to 37 %, with competitive ability depending on density in the field and weather conditions, particularly rainfall, during the growing period of the crop [184,185]. Saad et al. evaluated the allelopathic activity of *C. sinensis*, *C. limon*, *C. paradisi* and *C. aurantiifolia* EOs on seed germination and seedling growth of *S. marianum* [140]. The activity of *C. sinensis* EO has been also evaluated against *Anagallis arvensis* L. and *Malva parviflora* L., showing a growth inhibition of 76.5 % and 57.0 % at a concentration of 5 %, respectively [186]. Another study evaluated the effect of EO of *C. reticulata* and *C. aurantium* on the germination of *M. parviflora*, demonstrating a high efficacy in inhibiting the growth of this weed [187]. In particular, the EOs of *C. aurantium* (3 %) and *C. reticulata* (1 %) completely blocked the germination of mallow, confirming the potential of these extracts as natural bio-herbicides. Furthermore, EOs were tested against *Portulaca oleracea* L., a particularly resistant and problematic weed in agriculture. The results showed that the essential oil of *C. aurantium* (2 %) completely inhibited the germination and growth of purslane, suggesting a high allelopathic activity also on this species [187].

C. bergamia EO was tested to evaluate its efficacy against three weed species: *Amaranthus retroflexus* L., *Convolvulus arvensis* L., and *Rumex crispus* L. The concentrations used ranged from 5 to 20 μ L/mL. Under *in vivo* conditions, bergamot EO demonstrated significant toxicity to germinated seeds and young plants. At 24 h after application, the EO

caused weed mortality ranging from 33.3 % to 62.5 % [188].

Other studies reported the use of citrus extracts as herbicides or plant growth inhibitors (Table S9, Supplementary Material). *C. aurantiifolia* can inhibit the growth of *Lemna minor* L., known as duckweed, a free-floating green plant characterized by a wide range of growing conditions, resilience and adaptability. Additionally, *C. hystrix* has also been shown to inhibit the growth of duckweed [189]. Methanolic extract of *C. reticulata* peel was evaluated for germination and growth of *E. crus-galli*. The crude extract was applied at different concentrations to determine the degree of inhibition of seed germination. The results showed significant inhibition of germination of *E. crus-galli*, with a reduction of 79.07 % at the concentration of 2500 mg/100 mL [190]. Methanolic and aqueous extracts of *C. sinensis* were tested on the germination and growth of *Amaranthus hybridus* L. The results showed that the 50 % methanolic extract was the most effective in inhibiting germination, reducing it by 85.6 %, while the aqueous extract at the same concentration caused an inhibition of 77.8 %. In addition to the effect on germination, the extract significantly reduced shoot and root length, which determined the lower vigor of the seedlings [191]. The waste obtained from *Citrus x junos* Siebold ex Yu. Tanaka after juice extraction was extracted with methanol to analyze its effect on the germination and growth of different weed species, including *Digitaria sanguinalis* (L.) Scop., *Lolium multiflorum* Lam. and *Pheleum pratense* L. The methanolic extracts of the different parts of the fruit – peel, pulp and seeds – showed significant inhibitory activity, with a more marked effect attributed to the peel extract, followed by the pulp and finally the seeds [192]. Orange pulp aqueous extract, obtained by hydrodynamic cavitation, was tested on the germination of *Chenopodium album* L. Experiments were conducted under different conditions, including Petri dish and open field tests. The results showed that, in Petri dishes, OPWE almost completely inhibited the germination of common buckthorn at the highest concentrations, with a drastic reduction in germination already at 50 % dilution and a total inhibition at concentrations equal to or greater than 75 %. In open field conditions, the application of the extract led to a decrease in the presence of *C. album* and other weeds [193].

These studies further support the potential of citrus-derived compounds in plant growth inhibition and herbicidal applications.

4. Citrus bioactive compounds effective in crop protection

Citrus fruits are rich sources of antioxidant secondary metabolites, including terpenoids and phenolics. In addition to low-molecular terpenoids, limonoids and carotenoids are present in citrus fruits. Approximately 115 carotenoids have been identified in *Citrus* species. Their content and composition are influenced by factors such as variety, ripening, and environmental conditions. During ripening, carotenoid content increases as chlorophyll decreases. Studies suggest that citrus pulp contains similar or higher levels of bioactive carotenoids than fresh juice, highlighting the nutritional benefits of consuming pulp. Phenolics include flavonoids, phenolic acids, and coumarins [10]. Among *Citrus* flavonoids, flavanones are the most prevalent [194]. The most common flavanones are hesperidin, naringin, narirutin, neohesperedin, didymin, neoeriocitrin, eriocitrin and poncirin [195]. The levels and types of flavonoids vary between *Citrus* species and parts, influenced by factors such as plant age, fruit maturity, post-harvest treatments, and extraction methods. In *Citrus* fruits, flavonoids are mainly found in the peels, pulp, and seeds. However, peel residues are a rich source of these compounds, with higher levels than seeds [196]. Juicing and processing fresh fruit can reduce flavonoid content by 50 % [194]. The phenolic acid composition varies among *Citrus* fruits, depending on factors such as variety, part, and ripeness. For example, a study of the phenolic profiles in orange peel during the development of the fruit showed higher levels in the unripe and semi-mature stages compared to the commercial ripening stage [197]. *Citrus* peels are a precious source of phenolic compounds, including several hydroxycinnamic acids of relevance, such

as caffeic, p-coumaric, ferulic and sinapic. In particular, the extract obtained from the peel of the bitter orange (*C. aurantium*) stands out for the high concentration of these compounds, which represent approximately 73.8 % of the total content. Among these, p-coumaric acid and ferulic acid are the most present, with 24.68 % and 23.79 % respectively [198].

4.1. Antibacterial activity

Regarding antibacterial activity, the pure compounds tested are mostly EO components (Table S10, Supplementary Material). Xcc is the most devastating citrus disease worldwide, causing canker symptoms on all aerial parts of the plant [199]. The disease is characterized mainly by the formation of erumpent, corky and raised pustules on the surface of leaves, fruits and twigs, which serve as a source of bacterial inoculum [200]. Defoliation and fruit drop are also observed as a plant response to the infection [201]. There are few reports on the antibacterial activity of EO constituents against Xcc. Linalyl acetate, linalool, citral, citronellal, geraniol and α -terpineol were tested against Xcc. The lowest MIC value was related to citral (0.38 mg/mL), while the other tested compounds, linalyl acetate, citronellal, geraniol, linalool, α -terpineol and citral reported higher MIC value than *C. aurantifolia* EO with 0.50 mg/mL [85].

4.2. Antifungal activity

Extensive literature search revealed remarkable antifungal potential of some pure compounds isolated from *Citrus* species (Table S11, Supplementary Material). Their ability to inhibit and even eradicate fungal growth at low concentrations highlighted them as promising natural antifungal agents, suitable for applications in both agricultural and medical applications. The broad applicability of the citrus compounds in the fight against fungal pathogens is underlined by the variety of fungal species tested and the consistency of results across different methodologies. Carvacrol, a natural compound found in the EOs of various plants such as oregano and thyme, has been extensively studied for its potent antifungal properties. Several studies have demonstrated its efficacy against a variety of fungal species, using different test methods to assess its inhibitory capabilities. In research conducted by Sokovic et al., carvacrol was evaluated for its efficacy against *Verticillium fungicola* using the microatmosphere test, macrodilution test, and microdilution test [202]. The results showed a MIC range of 0.02–0.50 μ L/mL, indicating that very low concentrations of carvacrol are sufficient to inhibit the growth of these fungi. In addition, the MFC ranged from 0.05 to 0.25 μ L/mL, demonstrating the potential of carvacrol as a fungicidal agent at relatively low doses. Menthol was also tested against *V. fungicola*. The study showed that menthol effectively inhibited fungal growth, with a MIC and a MFC ranging between 0.25 and 1.50 μ L/mL. This indicates that menthol can suppress fungal growth at relatively low concentrations, highlighting its potential as a fungicidal agent. Further insight into the antifungal activity of carvacrol was provided by Pérez-Alfonso in 2012, who investigated its effects on *P. digitatum* and *P. italicum* using disc diffusion methods. The results were promising, with carvacrol showing 80 % growth inhibition against *P. digitatum* at a concentration of 500 μ L/L. More impressively, it achieved complete growth inhibition (100 %) against *P. italicum* at the same concentration, highlighting its broad-spectrum antifungal activity [203].

Several pure compounds have been evaluated for their ability to inhibit the growth of *P. digitatum*, a major fungal pathogen responsible for citrus fruit spoilage. While many of these compounds showed some antifungal activity, few achieved significant results, with thymol and octanal standing out for their high potency. Among the compounds tested, thymol showed complete inhibition of *P. digitatum* growth [203]. Similarly, octanal achieved 100 % growth inhibition in a food poisoning technique, demonstrating its remarkable antifungal efficacy [204]. Both compounds are remarkable for their ability to completely suppress the growth of this pathogen. Another molecule with the same activity

against *P. digitatum* is linalool. In the study by Shimada et al., linalool was tested using the agar diffusion method and no colonies of *P. digitatum* were detected [205]. Other compounds showed varying levels of efficacy but did not reach the same inhibiting level. Scoparone and quercetin, achieved growth inhibition rates of 69 % and 60 %, respectively, in *in vivo* tests [206]. Nobiletin, a polymethoxylated flavonoid, was reported to inhibit *P. digitatum* mycelial growth by 75 % [207]. Decanal, which inhibited growth by 70.56 %, was another compound that showed some promise but did not reach complete inhibition [204]. Lower levels of activity were observed with compounds such as scopoletin (40 % of growth inhibition) [206], hesperidin (38 %) [207], naringin (25 %) [207], and citral (24 %) [204]. In addition to these observations, further quantitative analysis allowed the determination of the IC₅₀ values of naringin and tangeretin, 10.4 g/L and 2.4 g/L, respectively [208].

More recently, nobiletin was reported to have the best inhibition rate against *B. cinerea*, *F. oxysporum* and *Sclerotinia sclerotiorum* at 0.1 mM, approximately 60 % [209]. Nobiletin was also tested against *C. gloeosporioides* using the poisoned technique, achieving 100 % activity at 100 μ g/mL. The same pathogen is also inhibited by nortangeretin (98 % of activity) [210].

Phytophthora citrophthora is a pathogenic oomycete that mainly affects citrus fruits but can also infect other ornamental and cultivated plants. The pure compounds isolated from *Citrus* fruit peel extracts were characterized and tested for their antifungal activity *in vitro*. Naringenin and hesperetin showed the highest activity, with IC₅₀ values of 0.19 mM and 0.21 mM, respectively, followed by nobiletin (IC₅₀ = 0.62 mM), sinensetin (IC₅₀ = 0.88 mM), heptamethoxyflavone (IC₅₀ = 1.06 mM) and tangeretin (IC₅₀ = 1.68 mM) [211].

The antifungal activity of limonene, menthol, menthone and thymol on the growth of *Fusarium verticelloides* was tested. Thymol showed the greatest antifungal activity, completely inhibiting fungal growth at concentrations of 500 μ L/L, while limonene and menthol required 1000 μ L/L to achieve the same result. On the other hand, menthone demonstrated an inhibitory activity of 75 % at 1000 μ L/L [212].

Coumarins are natural compounds that are widely distributed in many plants and play a key role in defense mechanisms against pathogens and environmental stress. Many coumarins act by inhibiting the growth of pathogenic microorganisms through mechanisms that include damaging cell membranes and interfering with essential metabolic processes. Some coumarins, such as scoparone and scopoletin, are synthesized in response to infection or stress. They accumulate in plant tissues as phytoalexins and contribute to plant resistance. A recent study investigated the antifungal activity of six coumarins isolated from lime peel against the pathogenic fungus *Colletotrichum* sp., responsible for anthracnose. Limettin and bergaptene, both individually and in combination, significantly inhibited mycelial growth and over 95 % of spore germination [213].

In addition to the pathogens already mentioned, other fungi, such as *A. parasiticus*, *A. flavus* and *Fusarium semitectum* were also tested in a study evaluating the antifungal activity of natural and enzymatically modified flavonoids [214].

4.3. Insecticidal activity

Limonene has been extensively tested for its insecticidal properties against major pests of cereals and stored products [131]. These pests include *T. castaneum* (red flour beetle), *S. granarius* (grain weevil), *L. bostrychophila* (bookworm), and *C. ferrugineus* (rusty grain beetle). These pests cause significant damage to stored products, resulting in post-harvest losses and posing a major threat to global food security. *C. maculatus* and *S. zeamais* are other two other important stored product pests. The study by Oyedeji et al. investigated the insecticidal activity of several pure constituents (e.g., limonene, geraniol, L-carvone, terpineol, linalool, citral, 3-carene, citronellol and β -caryophyllene) of *C. sinensis* peel against these pathogens. In the fumigant test, citral was the most

effective, with an LC₅₀ of 0.19 µL/L air for *C. maculatus* and 2.02 µL/L air for *S. zeamais* after 24 h exposure. Other monoterpenes such as limonene, terpineol, and linalool showed moderate fumigant activity, but less than citral. In the contact test, the results showed increased specific toxicity for some compounds. Terpineol was found to be the most effective against *C. maculatus*, with an LD₅₀ of 17.05 µg/adult, while for *S. zeamais* the most toxic compound was 3-carene, with an LD₅₀ of 26.01 µg/adult [136]. The activity of the two main enantiomers of limonene ((*R*)-limonene and (*S*)-limonene) against *S. zeamais* was evaluated by Fouad et al. (*R*)-limonene was the more promising of the two, due to its greater toxicity. However, both enantiomers showed good potential as natural pesticides [135].

Ceratitis capitata (Mediterranean fruit fly) and *T. urticae* (two-spotted spider mite) are notorious insect pests that cause extensive fruit losses worldwide. Interestingly, Papachristos et al. reported that the most potent compounds were *R*-(+) and *S*-(-) limonene, along with γ-terpinene, with LC₅₀ values ranging from 6.2 to 7 µL/g of food, followed by myrcene, which had an LC₅₀ of 9.6 µL/g. In contrast, the pinenes were the least effective, with significantly higher LC₅₀ values compared to other monoterpenes and sesquiterpenes. Oxygenated monoterpenes, including citral, linalool, α-terpineol, terpinen-4-ol, linalyl acetate, geranyl acetate, and neryl acetate, showed similar levels of activity, being 1.5 to 1.9 times more potent than the most active hydrocarbon. The toxicity of the hydrocarbon sesquiterpenes was slightly lower than that of hydrocarbon monoterpenes, with LC₅₀ values of 8.3 and 10.4 µL/g for caryophyllene and valencene, respectively [144]. In contrast, *T. urticae* was subjected to fumigant and contact assays, as detailed by Ribeiro et al. [141]. Limonene showed moderate efficacy with an LC₅₀ of 9.80 µL/L air and 76.97 µL/mL in the fumigant and contact assays, respectively. Eugenol proved to be one of the most potent compounds, with an LC₅₀ of 0.004 µL/L air and 8.73 µL/mL. Linalool showed moderate potency with an LC₅₀ of 1.94 µL/L air and 123.33 µL/mL. Similarly, α-terpineol was relatively potent with an LC₅₀ of 2.39 µL/L air and 43.27 µL/mL [141]. Another study about the activity of citral and limonene against *C. capitata* was conducted by Ruiz et al. Citral showed higher toxicity compared to limonene on eggs, while limonene showed comparable efficacy to citral against larvae. The activity of these two compounds was also evaluated against *A. fraterculus* [156]. Furthermore, in the study by Zarrad et al., limonene also showed excellent activity against *B. tabaci*. Fumigant tests showed a dose-dependent effect, with mortality increasing with increasing concentrations of limonene. At the highest concentration (20 µL/L air), it caused a 100 % mortality of adults within 24 h [147].

Detailed information on the activities of the pure compounds is provided in Table S12 (Supplementary Material).

4.4. Herbicidal activity

Citral was found to completely inhibit the growth of *P. minor* at 0.50 mg/mL. Strong herbicidal activity was observed, indicating the potential of citral on this species. Citral showed a significant inhibitory effect on *A. fatua* seed germination at a concentration of 0.10 mg/mL. At a higher concentration of 0.50 mg/mL, it caused a 42 % reduction in coleoptile (the first leaf sheath) growth. At the same concentration, root growth was drastically reduced by 81 %. This compound significantly inhibited *E. crus-galli* seed germination at a concentration of 0.25 mg/mL. When the concentration was increased to 0.75 mg/mL, coleoptile growth was reduced by 59 % and root growth was reduced by 66 %. In all three cases, citral significantly affected both seed germination and subsequent growth, demonstrating its potential as a natural herbicide [181]. In addition, limonene was also tested for its herbicidal activity against the same three weed species, but it was found to be less effective than citral and the crude essential oil (Table S13, Supplementary Material).

4.5. Antiviral activity

Tobacco Mosaic Virus (TMV) is an important plant virus that causes significant yield losses in solanaceous crops worldwide [215]. The study by Wang et al. reported a comprehensive evaluation of the protective, inactive and curative activities of reticine A against TMV using *Nicotiana glutinosa* L. (Table S14, Supplementary Material). Reticine A, an alkaloid isolated from the peel of the industrial crop *C. reticulata*, showed significant protective activity against TMV with better control efficacy at 100 µg/mL than benzothiadiazole, a commercial chemical inducer of systemic immunity in plants. Interestingly, 3 days after TMV inoculation, the protective effect of reticine A was superior to its inactive and curative effects, with protective efficacies of 46.7 %, 69.3 % and 73.4 % at 20, 100 and 500 µg/mL, respectively. The results indicated that reticine A is a promising natural inducer of plant immunity. It is capable of inducing long-lasting systemic resistance in distant plant tissues after initial exposure. Reticine A was suggested for direct use or as an active component in crude extract formulations for early field application. It also has potential as a lead compound for further refinement. In addition, this study highlights the potential use of *C. reticulata* peel, an abundant bioresource derived from citrus industry waste, in crop protection [216].

5. Citrus-based formulations

The exploitation of citrus by-products as a source of functional compounds and their use in agriculture has now become a promising field [217]. Common citrus-based formulations include cinnamon oil, citronella oil, clove oil, eucalyptus oil, lemongrass oil, mint oil, orange oil and many others. Citri-V™ is a vaporized blend of citrus EOs (orange: bergamot, 1:1 v/v) [76]. Prev-Am®, (Oro Agri, Cape Town, South Africa) contains 5 – 6 % orange oil as the active ingredient, with D-limonene being the major component [77].

Nanotechnology (including nanoparticles, nanocapsules, and nano-emulsions) offers a solution by developing novel delivery systems through the development of nanoformulations with enhanced active ingredient solubility and controlled release [218]. The characteristics and stability of these formulations depend on the physicochemical properties of the oil phase, including its solubility in water, chemical stability, polarity, density, viscosity, and interfacial tension [131]. In addition, the increasing stability of these formulations makes them suitable for applications in the agro-food sector [219]. For instance, the encapsulation of *C. aurantium* EO in chitosan nanoparticles allowed a gradual release of the oil itself, helping to preserve its antioxidant properties during the post-harvest period of mushrooms [220]. Citrus EOs have shown promise but face challenges such as high volatility, environmental degradation and low water solubility [221]. Incorporating EOs into nanoemulsions or encapsulating them into nanoparticles represents an effective approach to improve their stability and efficacy. In this context, Campolo et al. evaluated the insecticidal activity of different citrus EOs against eggs and larvae of the tomato borer *T. absoluta*, testing both emulsions and PEG nanoparticles containing EOs [222]. Laudani et al. conducted experiments to evaluate the aphicidal activity of a nano-insecticide based on *C. sinensis* EO. The product was tested in laboratory and field conditions. The results showed that even at moderate concentrations (4–6 %), aphid mortality exceeded 90 % after 48 h, with efficacy comparable to that of deltamethrin, a common synthetic insecticide. However, the highest concentrations showed significant phytotoxic effects on citrus plants. This suggests the need for a balance between efficacy and crop safety [223].

6. Extraction and valorization of citrus waste for sustainable crop protection

Over the years, the citrus processing industry has focused on the production of fruit juices and EOs. This industry generates

approximately 60 million tonnes of citrus waste per year worldwide, representing 50–60 % of organic waste [224]. Therefore, it is essential to investigate the feasibility of converting this waste into commercially viable commodities.

Citrus waste consists mainly of solid residues such as peels, pulp, and seeds, and semi-solid residues. This waste is used in the production of bio-sorbents, nanomaterials, and organic fertilizers, while green extraction processes are used to recover pectin, bioactive nutraceuticals, and EOs [225]. Citrus peel preparation typically involves washing, peeling, dehydration, grinding, sieving, and storage. Dehydration and grinding are key factors influencing extraction performance, with dehydration aiding phenol release but potentially affecting yield depending on drying methods and conditions. While studies show mixed results for fresh and dried peel extraction, the optimal particle size after grinding varies between 0.2 and 1.8 mm depending on the citrus species or variety, with no universal ideal size due to differences in extraction methods [226]. Seeds, which account for approximately 2 %–7 % of the total weight of citrus fruit, are typically unused by-products. They can be easily separated during the juice extraction process, and proper management of these seeds can help mitigate the significant issue of seed disposal [227].

Conventional solvent extraction is the most used method for extracting compounds from citrus and is influenced by factors such as solvent type, concentration, temperature, time, and extraction cycles. While methanol provides high yields, ethanol is generally recommended for sustainable and safe extractions. Optimal ethanol concentrations (80–85 %) and temperature-time combinations vary from study to study, with water added to solvents increasing extraction efficiency [226]. The polyphenolic extracts of *C. medica* and *C. bergamia* were obtained by maceration in 100 % ethanol for 24 h at 4 °C, starting from the peel and pulp. The extracts were subsequently tested against *Pectobacterium carotovorum*, highlighting an antibacterial action and reduced biofilm formation [228]. Improved extraction techniques could enhance the valorization of citrus by-products, resulting in higher-quality bioactives and increased revenue [229]. Soxhlet extraction has many advantages due to the use of minimal solvent and the automation of the process. However, due to the higher temperature, there is a risk of degradation of thermolabile compounds [230]. Botanical extracts of *C. aurantium* and *C. sinensis* were obtained using acetone, ethanol and water as extraction solvents. The plant material, consisting of seeds, leaves and peels, was dried in the shade for two weeks and then ground. The extraction was performed by the Soxhlet method, using 100 g of powder in 1 L of solvent for 3–5 h. The insecticidal activity was evaluated against *D. mangiferae*, a phytopathogenic insect of *Citrus* [158]. Newer methods such as microwave, ultrasound, and enzyme-assisted extractions have been developed to improve yields [231]. Ultrasound-assisted extraction (UAE) was employed to isolate bioactive compounds from *C. aurantium* peel, using petroleum ether as a solvent. The plant material, previously homogenized for 5 min, was subjected to three ultrasound cycles at room temperature, lasting 10 min each. The use of UAE has probably improved the extraction efficiency, facilitating the breakdown of the cell wall and the extraction of lipophilic compounds from the plant matrix. The study evaluated the insecticidal activity of the extract obtained from the peel of *C. aurantium* against two phytophages of agricultural importance, *B. oleae* and *C. capitata* [232].

Hydro-distillation of leaves or peels is used to obtain the EOs [233]. In addition, the exploration of solvent options with enhanced properties, such as supercritical fluids, has attracted considerable attention [234]. Recently, researchers have also begun to investigate alternative solvents, such as deep eutectic solvents (DES), for extracting bioactive compounds from citrus by-products. Their combined use with non-conventional extraction methods represents a promising avenue of research, although it is not yet been tested to extract bioactive compounds for phytosanitary defence [235].

To effectively extract targeted bioactive compounds, it's essential to select the optimal source, as compound content varies significantly

among *Citrus* species and their fruit tissue locations. On the other hand, it becomes clear that the distribution of bioactive compounds extracted from the citrus peel wastes is species-dependent [236].

7. Conclusions

Citrus species represents a highly promising natural alternative for crop protection due to their diverse array of bioactive compounds, that provide broad-spectrum activity against a wide range of pests, pathogens, weeds or environmental stresses. EOs and extracts derived from citrus fruits such as oranges, lemons, limes, grapefruits, and mandarins are rich in potent phytochemicals, that give citrus EOs and extracts significant antibacterial, antifungal, herbicidal, nematocidal, insecticidal and acaricidal properties. The antibacterial properties of citrus EOs and extracts make them effective against various plant pathogens, helping to control bacterial diseases and reduce crop losses. Their antifungal activity targets fungal pathogens responsible for diseases such as powdery mildew and root rot, inhibiting fungal growth and promoting plant health. In addition, citrus EOs and extracts can act as natural herbicides, controlling the growth of unwanted weeds and thereby increasing crop yields. Their nematocidal properties help control plant-parasitic nematodes, protecting root systems and improving overall plant vitality. The insecticidal and acaricidal properties of citrus provide a natural method of controlling pests such as aphids and spider mites, reducing the need for synthetic products. Although the wide range of bioactive compounds present in *Citrus* species have demonstrated significant antimicrobial potential against a variety of phytopathogens, their efficacy against phytopathogenic viruses remains an under-explored area of research. For the future, it is essential to develop studies aimed at clarifying the antiviral mechanisms of citrus secondary metabolites. The integration of extracts and EOs into sustainable agricultural practices offers several advantages. It reduces reliance on synthetic products, thereby minimising environmental impact and potential health risks. This natural approach supports more environmentally friendly crop management by promoting biodiversity and reducing chemical run-off. Using waste can also result in healthier plants with improved resistance to disease and pests, ultimately increasing yields and quality. By aligning with environmental conservation goals, *Citrus* bioactive compounds, extracts and EO contribute to sustainable land management and a greener agricultural future.

CRedit authorship contribution statement

Marta Lo Vetere: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Valeria Iobbi:** Writing – original draft. **Anna Paola Lanteri:** Validation, Methodology, Investigation, Data curation, Conceptualization. **Andrea Minuto:** Methodology, Conceptualization. **Giovanni Minuto:** Methodology. **Nunziatina De Tommasi:** Validation, Supervision, Conceptualization. **Angela Bisio:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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Data availability

No data was used for the research described in the article.

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