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Aniseed, *Pimpinella anisum*, as a source of new agrochemicals: Phytochemistry and insights on insecticide and acaricide development

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A R T I C L E I N F O

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A B S T R A C T

Pimpinella anisum L. (Apiaceae), known around the world as aniseed, is a widely cultivated crop, native of the sub-Mediterranean area. Its essential oil (EO) is exploitable in different fields such as food and beverages, pharmaceutics, cosmetics, and nutraceuticals. Regardless of the geographic origin, the EO exhibited consistent *trans*-anethole predominancy. Among the numerous biological properties exerted by aniseed EO, its antimicrobial, antifungal, insecticidal, and acaricidal effects have been extensively investigated for the formulation of biopesticides against larvae and adults of various pests and vectors. Hereafter, the published data on the insecticidal and acaricidal activity of aniseed EO and its major compounds on agricultural pests, stored-product pests, and arthropods of medical and veterinary interest is reviewed. For each study, the arthropod and the developmental stage on which the aniseed EO or the aniseed EO-based formulation were tested, the mode of action, the main constituents, and the exerted mortality, as well as the toxicity to non-target organisms and the possible sub-lethal effects are reported. The advantages of the possible use of aniseed EO as a biopesticide are analysed, as well as the current weaknesses and the critical points to be overcome to open the doors to the industrial utilization of Apiaceae EOs by the agrochemical industry.

1. Introduction

1.1. Distribution and agronomic practices

The anise or aniseed, *Pimpinella anisum* L., is an aromatic plant belonging to the family Apiaceae (Umbelliferae). Locally known with several other names, such as anis vert, anisoon, sweet cumin, yansoon, roomy, or saunf [1,2], this aromatic plant is native to Southwest Asia, Greece, Egypt [3] and India [2]. Anise cultivation dates back to Roman, Greek, and Egyptian times, when the fruits were employed for medical purposes [3,4]. Nowadays, its cultivation has widely expanded due to its several applications in food, beverages, and medicinal industries. Turkey, Mexico, Egypt, Italy, Spain, Syria, France, Brazil, South Africa, Latin America, Bulgaria, and Tunisia are all important aniseed producers, while Germany and India became the main exporters of this spice [3, 5–7]. The cultivation of aniseed requires sunshine and warm climates, although this plant may also thrive in areas where low temperatures do not exceed 160–180 days. The plant prefers fertile, or relatively rich, well-drained sandy loam soils, and requires regular care with sporadic weeding [4,5]. Bhuvaneshwari et al. [4] demonstrated that the simultaneous use of 80 kg ha^{-1} of nitrogen and 60 kg ha^{-1} of phosphorus and potassium led to improved yields in terms of number of leaves, plant height, total leaf area, seed yield, number of fruits per umbel, and size of the umbel. The traditional practice of aniseed farming involves ploughing the fields and adding fertilizer (manure) during autumn months, while sowing should be conducted in April for successful cultivation [8, 9]. The germination of the shoots starts after a month, and the vegetative growth is very swift following the development of the first leaves. The fruits are the most used part of the plant and are harvested by shaking the crop between August and September when they are still slightly damp and dark in colour [8].

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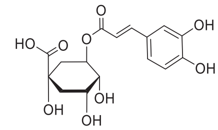
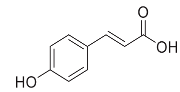
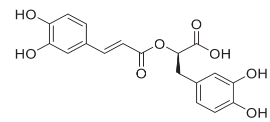
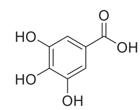
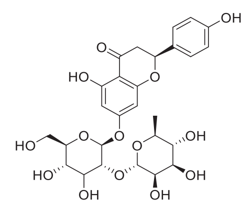
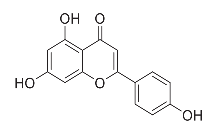
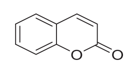
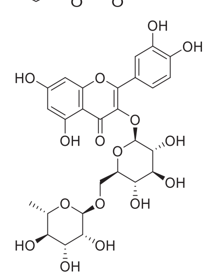
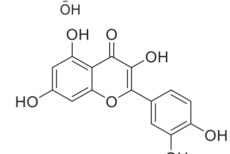
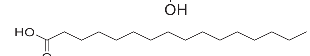
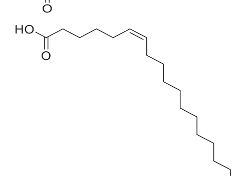
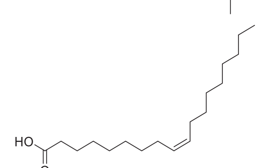
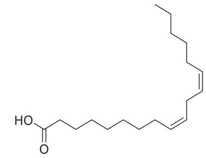
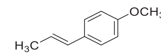
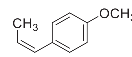
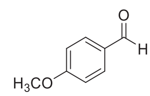
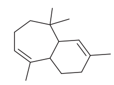
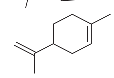
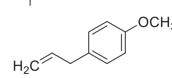
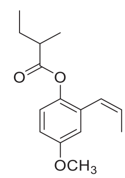
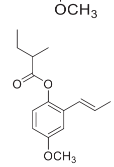
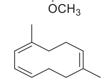
Table 1
Metabolites detected in *P. anisum*.

Class	Compounds	Structures	References
Phenolic acids	Chlorogenic acid		[40]
	p-Coumaric acid		
	Rosmarinic acid		
	Gallic acid		
Flavonoids	Naringin		[40]
	Apigenin		[41]
	Coumarin		
	Rutin		
	Quercetin		
Fatty acids	Palmitic acid		[40]
	Petroselinic acid		
	Oleic acid		

(*continued on next page*)

Table 1 (*continued*)

Class	Compounds	Structures	References
	Linoleic acid		
Volatile compounds	*trans*-Anethole		[7]
	cis-Anethole		
	p-Anisaldehyde		[153]
	γ-Himachalene		[154]
	Limonene		[55]
	Methyl chavicol		[153]
	cis-Pseudoisoeugenyl 2-methylbutirate		[7]
	trans-Pseudoisoeugenyl 2-methylbutirate		
	Pregeijerene		

1.2. Morphology and anatomy

P. anisum is an annual grassy herb which grows up to 30–50 cm with white flowers and small green to yellow seeds. The root primary state of growth lasts two weeks, while the secondary growth takes place in the 3rd and 4th weeks. Secondary phloem of older roots presents pericyclic secretory canals. Casparian thickenings can be noted in the endodermis, and the stems are often ribbed [10]. The leaves are dorsiventral, and the hairs are non-glandular and come in unicellular, dendroid, and stellate types. Secretory canals in the petiole and leaf lamina create a distinctive blend of oils, resin, and mucilage. The petiole is usually provided with an arc or ring of vascular bundles [11,12]. Flowers are terminal, small, bisexual, and epigynous. The sepals and calyx are absent. The corolla consists of five incurved petals, white in colour and distinct, with a retuse and valvate apex [13,14]. The fruit is a dry schizocarp, ovate and laterally compressed, consisting of two mericarps, each corresponding to one carpel containing one seed. The mericarp is about 3–5 mm long and 1.5–2 mm wide; it is ovoid-conical, greyish-brown, rough to the touch, and equipped with a series of vittae arranged in a circle to protect the seed. One mericarp is fertile, and the other is usually sterile. The fruit is orthospermous, i.e., the seeds contained in the carpels are flat on the inner surface, showing small dicotyledonous embryos at the apical end [15–17].

1.3. Traditional and medicinal uses

P. anisum fruits are traditionally used in many countries for the treatment of several diseases [18–20]. The first documented use of *P. anisum* fruits dates back to the 5th century in China, when they were used as an herbal remedy [21,22]. In ancient medical books, aniseed is reported as anti-asthma and anticonvulsant agent, and as a remedy for digestive disorders, dyspnea, and gynaecological problems [23]. In the Iranian traditional medicine, it is used as diuretic, carminative, and analgesic [24], and it is reported against melancholy, nightmares, seizure, and epilepsy in ancient texts [25,26].

Aniseed is part of the cultural experience of several countries, such as India [5], Palestine [27], Lebanon [28], Korea [29], and also European countries (*e.g.* United Kingdom and Italy) [6,30]. Notably, it is used for bronchial catarrh, pertussis, spasmodic cough, flatulent colic, insomnia, and constipation; externally, for pediculosis and scabies [29,31–33]. In Turkish medicine, it has an important role for its antifungal, antibacterial, and antiviral properties, as well as its anti-inflammatory, and hepato–protective activities [18,34,35].

1.4. Application in food, beverages, and cosmetic industries

For their pleasant odour and flavour, aniseed fruits acquired a great economic importance in food and beverages flavourings [36]. In some countries, *P. anisum* fruits are used for liquor, which is prepared with defined procedures and called with a specific name by each culture. Specifically, they derive by the distillation of dregs, grapes, and other fermented products, enriched with aniseed aroma. In the Mediterranean area, many aniseed spirit drinks like ouzo (Greek), anesone (Spain), pastis, and pernod (France), sambuca (Italy), zebib (Egypt), raki (Turkey), and arak (Syria) can be found [37]. Moreover, it is extensively

Table 2
Biological activities reported for *P. anisum* essential oil (EO).

Biological activity		Effect	References
Antibacterial	*Paenibacillus larvae*	MIC[a] of 300 μg/mL	[155]
	Streptococcus haemolyticus	inhibition zone (IZ) of 19 mm	[156]
	Staphilococcus aureus	MIC of 125.0 μg/mL	[157]
	Bacillus cereus	MIC of 62.5 μg/mL	
	Escherichia coli	MIC >500.0 μg/mL	
	Proteus vulgaris	MIC of 62.5 μg/mL	
	Proteus mirabilis	MIC of 125.0 μg/mL	
	Salmonella typhi	MIC of 500.0 μg/mL	
	Salmonella typhimurium	MIC of 250.0 μg/mL	
	Klebsiella pneumoniae	MIC >500.0 μg/mL	
	Pseudomonas aeruginosa	MIC >500.0 μg/mL	
	Bacillus thuringiensis	IZ of 15 mm	[158]
	Bacillus subtilis	IZ of 12 mm	
	native microflora of Swiss chard	MIC of 0.05 mL/100 mL	[159]
	Enterococcus faecalis	MIC of 4.88%	[160]
	Lactobacillus casei	MIC of 9.76%	
	Actinomyces naeslundii	MIC of 4.88%	
	Aggregatibacter actinomycetemcomitans	MIC of 9.76%	
Antifungal	*Aspergillus flavus*	IZ of 20 mm	[158]
	Trichoderma harzianum	complete IZ	
	Aspergillus niger	complete growth inhibition of the aggregate strain	[161]
	Aspergillus carbonarius	complete growth inhibition of the aggregate strain	
	Aspergillus parasiticus	reduced the biosynthesis of aflatoxin B1	[162]
	Pseudocercospora griseola	complete inhibition of conidal germination	[163]
	Fusarium oxysporum f.sp. *lycopersici*	MIC of 0.3 μL/mL of air	[164]
	Alternaria alternata	IZ increasing at increasing doses	[153]
	Candida albicans *Candida parapsilosis* *Candida tropicalis* *Candida pseudotropicalis* *Candida krusei* *Trichophyton rubrum* *Trichophyton mentagrophytes* *Microsporum canis* *Microsporum gypseum*	MIC of 0.10–0.78% (V/V)	[165]
Antiviral	PVX (potato virus) TMV (tobaccomosaic virus) TRSV (tobacco ring spot virus)	complete infection inhibition at 3000 ppm	[166]
Antioxidant	–	dose dependent DPPH[b] radical scavenging effect	[167]
Antiinflammatory	NF-κB mediated transcription in SW1353 cells	IC_{50} < 100 μg/mL	[168]
	inhibit the COX-2 expression	IC_{50} of 10.7 μg/mL	[169]
Anti-diabetic	rat jejunum	enhancement of glucose absorption	[28]
Anti-convulsant	rats	decreased hyperpolarization potential, increased firing frequency	[170]
		extended latency of seizure attacks reduced amplitude and duration of epileptiform burst discharges and dark neurons production	[171]
	male mice	suppressed tonic convulsions increased threshold of PTZ-induced clonic convulsion	[172]
Bronchodilatory	tracheal muscles of guinea pigs	relaxant effect	[23]
Estrogenic	YES[c] assay	EC_{50} of 570 μg/mL	[173]
Anticancer	HepG2 cell line	EC_{50} of 0.39 mg/mL	[59]
	MCF-7 cell line	EC_{50} of 0.25 mg/mL	
	Caco2 cell line	EC_{50} of 0.30 mg/mL	
	THP-1 cell line	EC_{50} of 0.11 mg/mL	
	A549 cell line	IC_{50} of 334.2 μg/mL	[81]
Palliation of nausea	patients (case study)	relief from the symptoms	[174]
Effect on morphine dependence	mice	induced conditioned place aversion and reduced morphine effect	[175]
Analgesic	mice	comparable to that of morphine and aspirin	[176]
Effect on broiler performance	day-old broilers	improved feed conversion ratio by approximately 6%	[177]
Influence on drug effects	mice	influenced effects of codeine, diazepam, midazolam, pentobarbital, imipramine, and fluoxetine on the central nervous system	[178]
	mice	significant decrease of plasma concentration of acetaminophen and caffein in mice	[179]

[a] MIC, Minimum Inhibitory Concentration.
[b] DPPH, 2,2-diphenyl-1-picrylhydrazyl.
[c] YES, yeast estrogen screen.

employed to produce teas and infusions due to the digestive and carminative properties of the plant.

1.5. Secondary metabolites

P. anisum is a source of several secondary metabolites, and its composition has been widely investigated. These compounds are distributed in all plant parts, but they are particularly concentrated in fruits inside the secretory structures (vittae) (Table 1). Generally, aniseed is rich in volatile compounds, phenolic compounds including flavonoids, and tannins [38,39]. Among phenolic compounds, chlorogenic, *p*-coumaric, rosmarinic, and gallic acids are the most abundant [40]. On the other hand, the flavonoids detected in aniseed extracts are naringin, apigenin, luteolin, rutin, and quercetin derivatives [40,41]. Phenolic acids and flavonoids have been demonstrated to be responsible for the antioxidant and antimicrobial activities of plant extracts [40]. The content of the above-mentioned secondary metabolites sensibly varies through geographical regions, culture conditions, harvesting time, storage, and manipulation procedures [42,43]. Concerning primary metabolites, aniseed contains fatty acids, such as petroselinic, oleic, and linoleic acids as the most abundant unsaturated fatty acids, and palmitic acid as the main saturated fatty acid [40] (see Table 1).

As mentioned above, *P. anisum* is characterized by a volatile fraction, represented by a fragrant EO, which is mainly composed of

Table 3
P. anisum EO activity evaluated against immature and adult stages of arthropods of medical and veterinary interest. In addition to the mortality rates, the mode of action and the percentage of main compounds are reported; n.a. = not available data.

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
Diptera	Culicidae	*Aedes aegypti*	adults	Vapor	n.a.	LC_{95} = 392.9 mg/mat (1 h)		[110]
			pupae	Aqueous solution	n.a.	3.84% (72 h)		[180]
			4th instar	Aqueous solution	n.a.	0.6% (24 h)		[180]
			larvae	Aqueous solution	n.a.	LD_{95} = 115.7 μg/mL (24 h)		[110]
			3rd instar larvae	Aqueous solution	commercial EO	LC_{50} = 0.023 ppm (24 h) LC_{50} = 0.020 ppm (48 h) LC_{90} = 0.043 ppm (24 h) LC_{90} = 0.037 ppm (48 h)	LC_{25} = 0.016 (24 h) LC_{25} = 0.014 (48 h)	[111]
			eggs	Aqueous solution	n.a.	EC_{95} = 34.3 μg/mL		[110]
Diptera	Culicidae	*Anopheles stephensi*	adults	Vapor	n.a.	LC_{95} = 378.5 mg/mat (1 h)		[110]
			4th instar larvae	Aqueous solution	n.a.	LD_{95} = 115.7 μg/mL (24 h)		[110]
			eggs	Aqueous solution	n.a.	EC_{95} = 33.3 μg/mL		[110]
Diptera	Culicidae	*Culex pipiens*	3rd/4th instar larvae	Aqueous solution	*(E)*-anethole (94.4%); methyl chavicol (2.7%); γ-himachalene (1.3%); *p*-anisaldehyde (0.3%); α-zingiberene (0.1%); γ-terpinene (0.1%); *p*-cymene (0.1%)	LC_{50} = 15.24 mg/L (24 h) LC_{90} = 23.79 mg/L (24 h)		[117]
			2nd/3rd instar larvae	Aqueous solution	Anethole (94.16%); *p*-allylanisole (2.77%); anisaldehyde (2.66%); γ-himachalene (0.41%)	LC_{50} = 28.7 ppm (48 h) LC_{90} = 49.5 ppm (48 h)		[181]
Diptera	Culicidae	*Culex quinquefasciatus*	adults	Tarsal contact	(*E*)-Anethole (97.9%); (*E*)-pseudoisoeugenyl 2-methyl butyrate (1.3%); methyl cavicol (0.6%); (*Z*)-anethole (0.1%)	LD_{50} > 200 μg/cm^2 (24 h)		[88]
				Tarsal contact	*trans*-Anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	LD_{50} = 0.6 μg/cm^2 LD_{90} = 1.2 μg/cm^2 LT_{50} = 12 min LT_{90} = 25 min	at 2 μg/cm^2	[114]
				Fumigation	*trans*-Anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	LD_{50} = 1.9 μL/L LD_{90} = 3.1 μL/L LT_{50} = 180 min LT_{90} = 226 min	at 10 μL/L (LC) at 4 μL/L (LT)	[114]
				Spray	*trans*-Anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	LC_{50} = 9.3 μL/mL LC_{90} = 25.1 μL/mL LT_{50} = 9 min LT_{90} = 35 min	at 50 μL/mL (LC) at 30 μL/mL (LT)	[114]
				Vapor	n.a.	LC_{95} = 354.9 mg/mat (1 h)		[110]
			pupae	Aqueous solution	*trans*-Anethole (78.0%), β-myrcene (15.3%), limonene (2.1%)	LC_{50} = 51.6 μg/mL (24 h) LC_{90} = 102.0 μg/mL (24 h)		[182]
			larvae	Aqueous solution	*trans*-Anethole (93.0%); methyl cavicol (15.0%); *p* – anisaldehyde (1.7%); γ-himachalene (1.5%)	LC_{50} = 25.4 μL/L (24 h) LC_{90} = 29.3 μL/L (24 h)		[46]

(*continued on next page*)

Table 3 (*continued*)

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
			4th instar larvae	Aqueous solution	n.a.	LD_{95} = 149.7 µg/mL (24 h)		[110]
				Aqueous solution	*trans*-Anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	LC_{50} = 26.1 µL/L LC_{90} = 30.1 µL/L LT_{50} = 235 min LT_{90} = 284 min	at 100 µL/L (LC) at 40 µL/L (LT)	[114]
			3rd instar larvae	Aqueous solution	*trans*-Anethole (78.0%), β-myrcene (15.3%), limonene (2.1%)	LC_{50} = 4.6 µg/mL (24 h) LC_{90} = 9.0 µg/mL (24 h)		[182]
				Aqueous solution	(*E*)-Anethole (97.9%); (*E*)-pseudoisoeugenyl 2-methyl butyrate (1.3%); methyl cavicol (0.6%); (*Z*)-anethole (0.1%)	LC_{50} = 25.9 µL/L (24 h) LC_{90} = 31.9 µL/L (24 h)		[88]
				Aqueous solution	*trans*-Anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	LC_{50} = 27.2 µL/L LC_{90} = 34.5 µL/L LT_{50} = 71 min LT_{90} = 167 min	at 100 µL/L (LC) at 40 µL/L (LT)	[114]
			2nd instar larvae	Aqueous solution	*trans*-Anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	LC_{50} = 26.6 µL/L LC_{90} = 34.1 µL/L LT_{50} = 15 min LT_{90} = 27 min	at 100 µL/L (LC) at 40 µL/L (LT)	[114]
			eggs	Aqueous solution	*trans*-Anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	n.d.	at 100 µL/L (LC)	[114]
				Aqueous solution	n.a.	EC_{95} = 33.8 µg/mL		[110]
Diptera	Muscidae	*Lucilia sericata*	3rd instar larvae	Ingestion	Commercial EO	LC_{50} = 2.74% LC_{90} = 24.68% LC_{95} = 46.04%		[183]
Diptera	Muscidae	*Musca domestica*	adults	Contact	*trans*-Anethole (68.76%); α-himachalene (11.88%); p-anisaldehyde (6.31%); estragole (3.42%); β-bisabolene (1.25%)	LC_{50} = 22.4 mg/dm^3	After 30 min	[184]
				Contact	*trans*-anethole (93.0%); methyl cavicol (15.0%); *p*–anisaldehyde (1.7%); γ-himachalene (1.5%)	LC_{50} = 54.8 µg/adult (24 h) LC_{90} = 99.7 µg/adult (24 h)		[46]
			pupae	Contact (topical)	n.a.	LC_{50} = 3.5% (10 days) LC_{90} = 8.7% after (10 days)		[180]
			3rd instar larvae	Aqueous solution	n.a.	LC_{50} = 11.4% (3 days) LC_{90} = 21.0% (3 days)		[180]
Mesostigmata	Dermanyssidae	*Dermanyssus gallinae*	adults	Contact	(*E*)-Anethole (94.8%); methyl chavicol (2.6%); (E)-pseudoisoeugenyl 2-methylbutyrate (1.3%); γ-himachalene (0.8%); germacrene D (0.2%)	LC_{50} = 47.5 µg/mL (24 h) LC_{90} = 121.9 µg/mL (24 h)		[185]
				Vapor	(*E*)-anethole (94.8%); methyl chavicol (2.6%); (E)-pseudoisoeugenyl 2-methylbutyrate (1.3%); γ-himachalene (0.8%); germacrene D (0.2%)	† <10% with open container † 55–60% with closed container		[185]
Sarcoptiformes	Pyroglyphidae	*Dermatophagoides farinae*	adults	Fumigation	*trans*-Anethole (79.3%); estragole (8.8%); *p*-anisaldehyde (2.9%); limonene (1.3%); α-pinene (1.1%); α-caryophyllene (1.1%)	LC_{50} = 9.11 µg/cm^2		[186]

(*continued on next page*)

Table 3 (*continued*)

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
Sarcoptiformes	Pyroglyphidae	*Dermatophagoides pteronyssinus*	adults	Fumigation	*trans*-Anethole (79.3%); estragole (8.8%); *p*-anisaldehyde (2.9%); limonene (1.3%); α-pinene (1.1%); α-caryophyllene (1.1%)	LC_{50} = 7.59 μg/cm^2		[186]
Anoplura	Pediculidae	*Pediculus humanus capitis*	adutls	Contact	Anise camphor (85.2%); cadina-1,4-diene (2.5%); estragole (1.8%); (+) spthulenol (0.5%); (+) carvone (0.4%); β-biabolene (0.3%)	KT_{50} = 45.37% at 0.25 mg/cm^3 KT_{50} = 37.34% at 0.5 mg/cm^3		[187]
				Vapor	n.a.	KT_{50} > 60 min at 60 μL		[188]
Hemiptera	Reduviidae	*Triatoma infestans*	4th instar larvae	Contact (topical)	(*E*)-Anethole (74%)	induces knock down or death		[189]
				Fumigation	(*E*)-Anethole (74%)	induces knock down or death		[189]
			eggs	Fumigation	(*E*)-Anethole (74%)	induces knock down or death		[189]

LC = lethal concentration; LD = lethal dose; LT = lethal time; KT = median lethal time; *trans*-anethole, (*E*)-anethole, anethole and anise camphor are synonyms. Methyl chavicol and estragole are synonyms. n.d. = not detected.

phenylpropanoids, being *trans*-anethole the major exponent of this chemical class. *p*-Anisaldehyde, methyl chavicol, *trans*-pseudoisoeugenyl 2-methylbutirate, *cis*-anethole, the terpenes pregeijerene and *γ*-himachalene are found in minor amounts. These compounds are the responsible for the multiple biological activities associated with the EO (Table 2). Notably, pregeijerene and pseudoisoeugenyl 2-methylbutirate are phytochemical markers for the genus *Pimpinella* [7].

2. Essential oil

2.1. Essential oil extraction

The EO of *P. anisum* is usually obtained from dried schizocarps by hydrodistillation (HD), which, together with steam distillation (SD), is a traditional extractive technique for EOs. These methods are based on the plant matrix contact with water (for HD) or steam (for SD) and, in both cases, the aqueous steam crosses the plant material and allows the transport of the volatile compounds inside an appropriate condenser [44]. Several studies reported the use of HD, also recommended by the European Pharmacopoeia [45], for the extraction of aniseed schizocarps in deionized water in a 1:10 or 1:20 plant/water ratio for generally 2 or 3 h. The EO, which is of a light-yellow colour, is generally obtained in a yield of 2.0% (w/w) estimated on a dry weight basis [46–48]. This yield value is exceeded in the case of the Italian 'Castignano ecotype' aniseed samples, for which the highest obtained yield was 5.5%, but also in the case of aniseed from Turkey (5.6%) and other Italian regions (4.3%) [8]. This noticeable variability in EO amount can be correlated with the changeable growing, pedoclimatic and storage conditions of aniseed before being harvested and then commercialized [8]. The effect of schizocarps pre-treatment with ultrasounds or microwaves has also been evaluated. In fact, Lotfy et al. [49] highlighted that both pre-treatments led to higher yields if compared with the traditional HD process, and the maximum yield (3.0% w/w) was achieved with 60% of ultrasonic power for 30 min. Moreover, microwave and ultrasound pre-treatments led to an increase in the phenylpropanoids (mainly represented by *trans*-anethole) content compared to the traditional HD.

Romdhane and Tizaoui [50] also reported the design of a pilot plant to test SD for the determination of the optimal operating conditions for *P. anisum* EO isolation on an industrial level. The EO was obtained in 2.55% w/w yield after 2.5 h of extraction, with a pressure of 200 kPa, and a steam flow rate of 6 kg h^{-1} [50]. Besides the above-mentioned traditional extractive techniques, microwave-assisted extraction (MAE) is also frequently performed [51,52]. For instance, Boumahdi et al. [53] carried out a MAE on *P. anisum* schizocarps and compared it with traditional HD. In terms of extraction yields, the HD process gave a higher EO yield than the MAE (3.30 and 2.81%, respectively). However, MAE led to lower time and energy consumption than HD (0.089 and 0.438 kWh/g of EO, respectively) [53].

2.2. Main constituents and chemotypes

P. anisum contains an EO dominated by the phenylpropanoid *trans*-anethole in percentage varying from 65.6 [54] to 96.9% [9]. Methyl chavicol, also called *p*-allylanisole or estragole, is another phenylpropanoid that has been reported as the most abundant EO compound only in the case of aniseed from Morocco (76.7%) and Yemen (85.3%) [55]. Some studies reported that methyl chavicol was found in percentages of 1.6 [56] and 9.8% [57], while others indicated the sesquiterpene *γ*-himachalene (from 2.1 to 8.3%) [9] as the second most representative constituent of aniseed EO. Other minor compounds are *α*-terpineol, linalool [3], *trans*-pseudoisoeugenyl-2-methylbutyrate [58,59], *cis*-anethole [60], and anisaldehyde [61]. *Pimpinella anisum* EO yield and chemical composition are affected by several factors, including the geographical origin, the growing, pedoclimatic, and harvesting conditions, and the extraction methods and parameters. Orav et al. [7] investigated the EO composition of aniseed from different European countries, highlighting the highest contents of *trans*-anethole in EO from Hungary, Greece, Scotland, Lithuania, Italy, and Germany, while those from Estonia and Russia were particularly rich in *γ*-himachalene. Moreover, EOs from Estonia and France presented a significant amount of pseudoisoeugenyl-2-methylbutyrate and anisaldehyde [7]. Khalid [54] evidenced the importance of nitrogen and phosphorous micronutrients application, especially in desert areas, to enhance aniseed EO yield and content of the major compounds [54]. A higher amount of EO was also produced at lower plant densities and in wider row spacing [62]. Moreover, *P. anisum* should be sown in the early spring, especially in April, and seeds should be harvested at the waxy stage to obtain a higher

Table 4
Main components of aniseed oil insecticidal and acaricidal activity evaluated against immature and adult stages of arthropods of medical and veterinary interest. In addition to the mortality rates, the mode of actions is reported; n.a. = not available data.

Compound	Order	Family	Targeted species	Stage	Mode of action	Mortality rates or LC/LD		Notes	References
trans-anethole	Diptera	Culicidae	*Aedes aegypti*	adults	contact	LC_{50} = 0.003 mg/mL LC_{90} = 0.57 mg/mL			[190]
				pupae	Aqueous solution	LT_{50} = 163.2 h LT_{90} = 247.8 h	† 0.8% - dose 0.5%	Extracted from *Illicium verum* (star anise). Mortality (†) was checked (72 h).	[103]
						LT_{50} = 142.5 h LT_{90} = 218.1 h	† 3.2% - dose 1%		
						LT_{50} = 47.9 h LT_{90} = 58.1 h	† 52.6% - dose 2.5%		
						LT_{50} = 6.9 h LT_{90} = 11.5 h	† 100% - dose 5%		
				4th instar larvae	Aqueous solution	LT_{50} = 16.7 h LT_{90} = 24.9 h	† 8% - dose 0.5%	Extracted from *Illicium verum* (star anise) Mortality (†) was checked after 6 h.	[103]
						LT_{50} = 2.1 h LT_{90} = 4.9 h	† 70.8% - dose 1%		
						LT_{50} = 0.4 h LT_{90} = 0.7 h	† 97.5% - dose 2.5%		
						LT_{50} = 0.2 h LT_{90} = 0.4 h	† 100% - dose 5%		
	Diptera	Culicidae	*Aedes albopictus*	pupae	Aqueous solution	LT_{50} = 207.7 h LT_{90} = 2967.7 h	† 2.4% - dose 0.5%	Extracted from *Illicium verum* (star anise)	[103]
						LT_{50} = 174.5 h LT_{90} = 232.7 h	† 2.6% - dose 1%		
						LT_{50} = 57.6 h LT_{90} = 60.4 h	† 41.7% - dose 2.5%		
						LT_{50} = 28.8 h LT_{90} = 55.8 h	† 86.4% - dose 5%		
				4th instar larvae	Aqueous solution	LT_{50} = 13.4 h LT_{90} = 18.4 h	† 1.6% - dose 0.5%	Extracted from *Illicium verum* (star anise)	[103]
						LT_{50} = 13.3 h LT_{90} = 18.7 h	† 1.6% - dose 1%		
						LT_{50} = 0.5 h LT_{90} = 0.8 h	† 86.4% - dose 2.5%		
						LT_{50} = 0.3 h LT_{90} = 0.6 h	† 100% - dose 5%		
	Diptera	Culicidae	*Culex pipiens*	3rd/4th instar larvae	Aqueous solution	LD_{50} = 16.56 mg/L (24 h) LD_{90} = 25.29 mg/L (24 h)			[117]
	Diptera	Culicidae	*Culex quinquefasciatus*	adults	Contact	LD_{50} = 0.4 μg/cm^2 LD_{90} = 1.0 μg/cm^2		at 2 μg/cm^2	[114]

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Table 4 (*continued*)

Compound	Order	Family	Targeted species	Stage	Mode of action	Mortality rates or LC/LD	Notes	References
					Fumigation	LC_{50} = 2.1 μL/L LC_{90} = 3.3 μL/L	at 10 μL/L	[114]
					Spray	LC_{50} = 8.1 μL/L LC_{90} = 22.5 μL/L	at 50 μL/L	[114]
				pupae	Aqueous solution	LD_{50} = 28.6 μg/mL (24 h) LD_{90} = 48.6 μg/mL (24 h)		[182]
				4th instar larvae	Aqueous solution	LD_{50} = 19.8 μL/L LD_{90} = 31.3 μL/L	at 100 μL/L	[114]
				3rd instar larvae	Aqueous solution	LD_{50} = 21 mg/L (24 h) LD_{90} = 34 mg/L (24 h)	**synergistic** → p-cymene, γ-terpinene, eugenol, isoeugenol, L-carvone, (+)-limonene, α-pinene, β-citronellol, carvacrol, thymol, α-terpinene, (+)-camphor, (−)-borneol, cinnamyl alcohol, (−)-camphene, terpinolene, 4-allylanisole, α-terpineol, myrcene, menthone, cinnamaldehyde **no effect** →1,8-cineole, linalool, (±)-citronellal, (−)-β-pinene, trans-cinnamic acid, vanillin, dimethyl sulfide **antagonistic** → gallic acid	[113]
					Aqueous solution	LD_{50} = 7.4 μg/mL (24 h) LD_{90} = 18.8 μg/mL (24 h)		[182]
				3rd instar larvae	Aqueous solution	LD_{50} = 18.5 μL/L LD_{90} = 28.2 μL/L	at 100 μL/L	[114]
					Aqueous solution	LD_{50} = 24.8 μL/L (24 h) LD_{90} = 32.1 μL/L (24 h)		[88]
				2nd instar larvae	Aqueous solution	LD_{50} = 15.3 μL/L LD_{90} = 25.1 μL/L	at 100 μL/L	[114]
				eggs	Aqueous solution	† 33%	at 100 μL/L	[114]
	Diptera	Muscidae	*Musca domestica*	adults	Contact	LD_{50} = 20.5 mg/dm^3 after 30min		[184]
					Fumigation	† 54% - dose 0.5% (24 h) † 56.1% - dose 1.0% (24 h)	Extracted from *Illicium verum*	[100]
	Blattodea	Blattellidae	*Blattella germanica*	adults	Contact	† 100% - dose 0.199, 0.159, 0.099, 0.049 mg/cm^2 (♂) † 100% - dose 0.199 mg/cm^2 (♀) † 76.7% - dose 0.159 mg/cm^2 (♀) † 33.3% - dose 0.099 mg/cm^2 (♀) † 3.3% - dose 0.049 mg/cm^2 (♀)	Extracted from *Illicium verum*	[97]
					Contact	† 100% - dose 1.0 mg/adult (♂) † 100% - 0.5 mg/adult (♂) † 96.0% - 0.25 mg/adult (♂) † 6.0% - dose 0.125 mg/adult (♂) † 100% - dose 1.0 mg/adult (♀) † 96.0% - dose 0.5 mg/adult (♀) † 14.0% - dose 0.25 mg/adult (♀)		[191]
					Fumigation	† 100% - dose 20, 10 and 5 mg/filter (♂)		[191]

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Table 4 (*continued*)

Compound	Order	Family	Targeted species	Stage	Mode of action	Mortality rates or LC/LD	Notes	References
						† 77.5% - dose at 2.5 mg/filter (♂) † 7.5% - dose 1.25 mg/filter (♂) † 100% - dose 20 and 10 mg/filter (♀) † 82.5% - dose 5 mg/filter (♀) † 15.0% - dose 2.5 mg/filter (♀)		
	Ixodida	Ixodidae	*Dermacentor nitens*	nymphs	Contact	† 7.3% - dose 2.5 μL/L † 12.0% - dose 5.0 μL/L † 52.3% - dose 10.0 μL/L † 91.5% - dose 15.0 μL/L † 100% - dose 20.0 μL/L		[192]
	Ixodida	Ixodidae	*Rhipicephalus annulatus*	adults	Contact	LC_{50} = 2.36% LC_{90} = 5.49%	Extracted from *Foeniculum vulgare*	[99]
	Ixodida	Ixodidae	*Rhipicephalus microplus*	adults	Contact	† 0% - dose 2.5 μL/L † 73.4% - dose 5.0 μL/L † 71.8% - dose 10.0 μL/L † 95.9% - dose 15.0 μL/L † 100% - dose 20.0 μL/L		[192]
	Anoplura	Pediculidae	*Pediculus humanus capitis*	adults	Vapor	KT_{50} > 60min at 60 μL		[188]
	Hemiptera	Reduviidae	*Triatoma infestans*	4th instar nymphs	Contact	LD_{50} = 0.26 mg/cm^2		[189]
				1st instar nymphs	Contact	LD_{50} = 0.83 mg/cm^2		[189]
				eggs	Contact	LC_{50} > 2 mg/cm^2		[189]
3-carene	Sarcoptiformes	Pyroglyphidae	*Dermatophagoides farinae*	adults	Fumigation	LC_{50} = 42.10 μg/cm^2		[186]
	Sarcoptiformes	Pyroglyphidae	*Dermatophagoides pteronyssus*	adults	Fumigation	LC_{50} = 39.84 μg/cm^2		[186]
β-myrcene	Blattodea	Blattellidae	*Blattella germanica*	adults	Contact	† 42.0% - dose 1.0 mg/adult † 18.0% - dose 0.5 mg/adult (♂) † 34.0% - dose 1.0 mg/adult (♀) † 5.0% - dose 20 mg/filter (♂) † 2.5% in - dose 20 mg/filter (♀)		[191]
	Anoplura	Pediculidae	*Pediculus humanus capitis*	adults	Vapor	KT_{50} = 48.90 min at 60 μL		[188]
	Diptera	Culicidae	*Culex quinquefasciatus*	pupae	Aqueous solution	LC_{50} = 74.8 μg/mL (24 h) LC_{90} = 155.0 μg/mL (24 h)		[182]
				3rd instar larvae	Aqueous solution	LD_{50} = 14.2 μg/mL (24 h) LD_{90} = 36.4 μg/mL (24 h)		[182]
limonene	Blattodea	Blattellidae	*Blattella germanica*	adults	Contact	† 40.0% - dose 1.0 mg/adult † 20.0% - dose 0.5 mg/adult (♂) † 26.0% - dose 1.0 mg/adult (♀) † 85.0% - dose 20 mg/filter † 17.5% - dose 10 mg/filter (♂) † 75.0% - 20 mg/adult		[191]

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Table 4 (*continued*)

Compound	Order	Family	Targeted species	Stage	Mode of action	Mortality rates or LC/LD	Notes	References
						† 10.0% - 10 mg/filter (♀)		
	Anoplura	Pediculidae	*Pediculus humanus capitis*	adults	Vapor	KT_{50} = 27.20 min at 60 μL		[188]
	Diptera	Culicidae	*Culex quinquefasciatus*	pupae	Aqueous solution	LD_{50} = 31.8 μg/mL (24 h) LD_{90} = 59.1 μg/mL (24 h)		[182]
				3rd instar larvae	Aqueous solution	LD_{50} = 19.5 μg/mL (24 h) LD_{90} = 40.0 μg/mL (24 h)		[182]
	Diptera	Culicidae	*Aedes aegypti*	pupae	Aqueous solution	LD_{50} = 3.7%	ex *Z. limonella*	[103]
				4th instar larvae	Aqueous solution	LD_{50} = 2.9%	ex *Z. limonella*	[103]
	Diptera	Culicidae	*Aedes albopictus*	pupae	Aqueous solution	LD_{50} = 3.7%	ex *Z. limonella*	[103]
				4th instar larvae	Aqueous solution	LD_{50} = 3.1%	ex *Z. limonella*	[103]
estragol	Sarcoptiformes	Pyroglyphidae	*Dermatophagoides farinae*	adults	Fumigation	LC_{50} = 43.23 μg/cm^2		[186]
	Sarcoptiformes	Pyroglyphidae	*Dermatophagoides pteronyssinus*	adults	Fumigation	LC_{50} = 40.11 μg/cm^2		[186]
p-anisaldehyde	Sarcoptiformes	Pyroglyphidae	*Dermatophagoides farinae*	adults	Fumigation	LC_{50} = 1.11 μg/cm^2		[186]
	Sarcoptiformes	Pyroglyphidae	*Dermatophagoides pteronyssinus*	adults	Fumigation	LC_{50} = 0.98 μg/cm^2		[186]

LC = lethal concentration; LD = lethal dose; LT = lethal time; KT = median lethal time; *trans*-anethole, (*E*)-anethole, anethole and anise camphor are synonyms. Methyl chavicol and estragole are synonyms. n.d. = not detected.

EO yield and content of *trans*-anethole [63]. A lack of water during stem elongation and umbel appearance decreased the EO production [64].

Genetic variations among plants belonging to the same species (chemotypes) can result in the biosynthesis of different chemical constituents, leading to widely diverse EO types, in terms of composition and bioactivity [65,66]. In *P. anisum*, the enzyme S-adenosyl-L-methionine:anol-O-methyltransferase (OMT) was demonstrated to directly participate in the development of the chemotype containing *trans*-anethole. Moreover, several genes appeared to be involved in the different biosynthetic pathways of either *trans*-anethole and methyl chavicol [67]. In general, according to the European Pharmacopoeia, a good aniseed chemotype is characterized by more than 90% *trans*-anethole and less than 1% methyl chavicol, because the latter was removed from the list of flavours in food stuffs, due to its harmful effect on animals. Notably, the populations with higher amounts of γ-himachalene and lower levels of methyl chavicol are considered as sweeter accessions, which can be employed in food products [68]. Additionally, significant differences in the chemical profiles of aniseeds and roots EOs can be observed. In fact, *trans*-epoxypseudoisoeugenyl-2-methylbutyrate, β-bisabolene, and pregeijerene were detected as the predominant components of the EO from *P. anisum* roots [69].

2.3. Applications and patents

Aniseed EO plays a key role in food technology since it can be used as a flavouring agent in several products, including bread, cakes, candies, and beverages [70]. The highest levels of this product accepted by FDA (Food and Drugs Administration) are 750 ppm for alcoholic beverages, and 680 ppm for candies. Moreover, the EO, which is endowed with a great antioxidant activity for the presence of high amounts of *trans*-anethole, can be employed as an additive [71] and to prevent food degradation. For this reason, it has an important economic impact due to the current demand for biological foods [38,42,72,73]. Aniseed is in fact 'generally recognised as safe' (GRAS) in the USA, and as a natural source of feed flavoring by the Council of Europe [74]. In several Mediterranean countries, traditional alcoholic beverages are produced with *P. anisum* thanks to the solubility of the EO and its main compounds in ethanol [75]. However, the development of non-alcoholic beverages with this product remains difficult since its constituents are insoluble in water. In this respect, nanotechnology may represent a new alternative to prepare non-alcoholic beverages with significant amounts of EO and their components, by employing nanoparticles, nanocapsules, nanodispersions, and nanoemulsions [76]. Nanotechnology would also offer other advantages to the food industry, through the development of safe products with considerably low toxicity, improved bioavailability of functional foods, and activity of preservatives [77]. Notably, the encapsulation of *P. anisum* EO was successfully performed into chitosan nanomatrix in the form of a nanoemulsion for the protection of stored rice against fungal-mediated biodeterioration [65]. In addition, aniseed EO was employed in different concentrations into gelatin–alginate coating for treating zucchini fruit to be used as an active edible coating able to extend the shelf life of this product during storage [78].

2.4. Toxicity and safety

The investigation of the toxicity and safety of botanical products is of crucial importance for their development and exploitation in several industrial fields. In this regard, the toxicity of *P. anisum* EO was evaluated on different cell lines. For instance, it was tested on Hep G2 cells, which are usually employed as *in vitro* alternatives to primary human hepatocytes [79], leading to significant cytotoxicity at increasing concentrations. In detail, it caused a 34 and 58% cell viability reduction at concentrations of 1.2 and 1.6% without an apoptotic/necrotic mechanism [60]. Aniseed EO was also tested on mouse fibroblasts (L929),

Table 5
P. anisum EO activity evaluated against immature and adult stages of stored product pests. In addition to the mortality rates, the mode of action and the percentage of main compounds are reported; n.a. = not available data.

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
Lepidoptera	Pyralidae	*Ephestia kuehniella*	adults	Fumigation	n.a.	at 4 µL/l air, † 26.3% (96 h); at 8 µL/l air, † 31.7% (96 h); at 16 µL/l air, † 45.0% (96 h); at 32 µL/l air, † 50.0% (24 h); at 64 µL/l air, † 100% (24 h)		[193]
			1st instar larvae	Vapor		at 135 µL/l, max 67.5% after 6 h; at 108 µL/l, † 58.3% after 9 h; at 54 µL/l, † 65.8% after 12 h; at 27 µL/l, † 63.3% (24 h); at 108 µL/l, † 98.3% (24 h)		[121]
				Contact		LC_{50} = 20.92% (24 h) $LC_{99.9}$ = 21.42% (24 h)		[193]
			eggs	Fumigation	n.a.	at 20 µL/l air, † 32.7% (24 h)		[194]
				Vapor	n.a.	LT_{99} = 60.9 h		[120]
Lepidoptera	Pyralidae	*Plodia interpunctella*	eggs	Fumigation	Commercial EO	† 28.7% - 20 µL/L air (24 h)		[194]
Coleoptera	Silvanidae	*Oryzaephilus surinamensis*	adults	Fumigation	n.a.	† 12% - dose 15 µL/L and 10 µL/L (24 h) † 7% - dose 5 µL/L (24 h)		[195]
Coleoptera	Curculionidae	*Sitophilus granarius*	adults	Treated wheat	Commercial EO	0.391 mL/kg (7 days)		[196]
				Contact	Anethole (88.6%)	† 30% - dose 2.5 µL (48 h) † 12.5% - dose 5.0 µL (48 h) † 22.0% - dose 9.5 µL (48 h) † 25.0% - dose 14 µL (48 h)		[197]
				Fumigation	Anethole (88.6%)	† 40.58% (24 h) † 61.08% (48 h) † 68.67% (72 h) at 1.97 µL/mL air † 64.5% (24 h) † 87.0% (48 h) † 94.67% (72 h) at 5.91 µL/mL air		[197]
Coleoptera	Curculionidae	*Sitophilus oryzae*	adults	Treated wheat	Commercial EO	LC_{50} = 0.515 ppm LC_{90} = 3.245 ppm		[198]
				Vapor	Anethole (832.46 mg/mL); 1,8-cineole (3.56 mg/mL); carvacrol (2.46 mg/mL)	25.8% (6 h) 36.6% (9 h) 32.5% (12 h) 95% (24 h) at 135 µL/L 50% (24 h) at 54 µL/L		[121]
				Fumigation	n.a.	† 40% - dose 15 µL/L (24 h) † 0% - dose 10 µL/L (24 h) † 10% - 5 µL/L (24 h)		[195]

(continued on next page)

Table 5 (*continued*)

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
				Fumigation	*E*-Anethole (76.56 mg/mL); estragol (13.01%); linalool (7.42%)	LC_{50} = 292.04 μL/L air (72 h) LC_{95} = 1281.12 μL/L air (72 h)		[199]
Coleoptera	Tenebrionidae	*Tenebrio molitor*	larvae	Contact	Anethole 88.6%	No toxicity observed		[197]
				Fumigation	Anethole 88.6%	No toxicity observed		[197]
Coleoptera	Tenebrionidae	*Tribolium castaneum*	adults	Contact	*trans*-Anethole (91.4%); estragole (3.4%); γ-himachalene (2.3%)	† 60.7% - dose 1.0 g/kg (14 days) † 72.0% - dose 2.5 g/kg (14 days) † 60.7% - dose 5.0 g/kg (7 days)		[200]
				Contact + Ingestion	E-Anethole (801 mg/g); limonene (55.7 mg/g); a-himachalene (25.2 mg/g); trans-verbenol (24.7 mg/g); linalool (16.4 mg/g); acethyl-isoeugenol (11.3 mg/g)	LD_{50} = 2.1% (v/v) (94 h)		[93]
				Contact+ Fumigation	*trans*-Anethole (91.4%); estragole (3.4%); γ-himachalene (2.3%)	† 67.7% - dose 0.25 mL/cm^2 (14 days) † 77.0% - dose 0.50 mL/cm^2 (14 days) † 55.0% - dose 1.00 mL/cm^2 (7 days) † 70.9% - dose 1.50 mL/cm^2 (7 days)		[200]
				Fumigation	*E*-Anethole (76.56 mg/mL); estragol (13.01%); linalool (7.42%)	LC_{50} = 43.75 μL/L (24 h) LC_{95} = 72.98 μL/L (24 h)		[199]
				Fumigation	*trans*-Anethole (84.1%); methyl-chavicol (2.54%); *p*-cymene (0.01%)	† 16.7% - dose 4 μL/L air (96 h) † 25.0% - dose 8 μL/L air (96 h) † 33.7% - dose 16 μL/L air (96 h) † 50.0% - dose 32 μL/L air (48 h) at 64 μL/L air, † 65% (24 h) at 128 μL/L air, † 100% (24 h)		[193]
				Fumigation	n.a.	† 100% - dose 15 μL/L (24 h) † 100% - dose 10 μL/L (24 h) † 0% dose 5 μL/L (24 h)		[195]
			1st instar larvae	Contact	*trans*-Anethole (84.1%); methyl-chavicol (2.54%); *p*-cymene (0.01%)	LC_{50} = 21.42% (24 h) LC_{99} = 40.85% (24 h)		[193]
Coleoptera	Tenebrionidae	*Tribolium confusum*	adults	Vapor	Anethole (832.46 mg/mL); 1,8-cineole (3.56 mg/mL); carvacrol (2.46 mg/mL)	max 10% - dose 135 μL/L (6–9 h) 15.8% - dose 135 μL/L (12 h) 95% - dose 81 μL/L (24 h)		[121]
			eggs	Fumigation	Commercial EO	LC_{50} = 20.42 μL/L air LC_{90} = 33.49 μL/L air		[194]
				Vapor	n.a.	LT_{99} = 253.0 h at 98.5 μL/L		[120]
Coleoptera	Tenebrionidae	*Trogoderma granarium*	adults	Contact/ Ingestion	(E)-Anethole (93%); p-anysaldehyde (1.8%); γ-himachalene (1.5%); methyl chavicol (1.5%); α-zingiberene (0.3%)	† 66.7% - dose 500 ppm (1 day) † 51.1% - dose 1000 ppm (16 h)		[201]
				Contact	*trans*-Anethole (91.4%); estragole (3.4%); γ-himachalene (2.3%)	† 60.3% - dose 1.0 g/kg (14 days) † 77.7% - dose 2.5 g/kg (14 days)		[200]

(*continued on next page*)

Table 5 (*continued*)

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
				Contact+ Fumigation	*trans*-Anethole (91.4%); estragole (3.4%); γ-himachalene (2.3%)	† 56.1% - dose 5.0 g/kg (7 days) † 67.0% - dose 0.25 mL/cm^2 (14 days) † 83.9% - dose 0.50 mL/cm^2 (14 days) † 60.0% - dose 1.0 mL/cm^2 (7 days) † 54.0% - dose 1.50 mL/cm^2 (7 days)		[200]
			larvae	Contact/ Ingestion	(E)-Anethole (93%); p-anysaldehyde (1.8%); γ-himachalene (1.5%); methyl chavicol (1.5%); α-zingiberene (0.3%)	† 65.6% - dose 500 ppm (1 day) † 66.7% - dose 1000 ppm (16 h)		[201]
Coleoptera	Chrysomelidae	*Callosobruchus maculatus*	adults	Contact	*trans*-anethole (86.74%); estragole (4.08%); methyl chavicol (1.68%)	LC_{50} = 4.9 mg/L (24 h) LC_{50} = 3.7 mg/L (48 h) LC_{50} = 2.5 mg/L (72 h)		[202]
				Fumigation	*trans*-Anethole (86.74%); estragole (4.08%); methyl chavicol (1.68%)	LC_{50} = 50.0 mg/L (24 h) LC_{50} = 3.7 mg/L (48 h) LC_{50} = 32.34 mg/L (72 h)		[202]
				Treated cowpea	Commercial EO	LC_{50} = 1.09 ppm LC_{90} = 6.82 ppm	† of 66.6% and 85.0% at LC50/LC90 with EO (3 days), 80.0% and 95.0% after 7 d † of 60.0% and 85.0% at LC50/LC90 with powder (3 days), 75.0% and 91.3% after 7 d	[198]

LC = lethal concentration; LD = lethal dose; LT = lethal time; KT = median lethal time; *trans*-anethole, (*E*)-anethole, anethole and anise camphor are synonyms. Methyl chavicol and estragole are synonyms. n.d. = not detected.

resulting not cytotoxic at the tested concentrations (i.e., 20, 8, 4 and 2 mg/mL) [80], and on human foetal skin fibroblast cells (WRL-68) with an IC_{50} of 334.2 μg/mL (dosages concentration 400 to 6.25 μg/mL) [81]. It was also assayed on brine shrimp larvae (*Artemia salina* L.), which are usually employed for cytotoxicity studies. In this regard, the study of Khafagi et al. [82] reported a LC_{50} higher than 1000 μL/mL, while that of Martins et al. [83] a LC_{50} of 293.8 μg/mL, classifying the EO as non-toxic. However, Ghosh et al. [84] reported IC_{50} values for *P. anisum* EO of 2.86–3.06 μg/mL on brine shrimp larvae, and these results are in contrast with the above-mentioned works.

Regarding *P. anisum* EO safety, this product is listed as GRAS by the FDA [85]. This classification relies on its low intake as flavouring agent (54 mg/kg body weight/day), metabolic detoxication in humans, low genotoxic or mutagenic potential, No Observed Adverse Effect Level (NOAEL) of 120 mg/kg body weight/day, and its low impact on the increase of hepatocellular tumours. According to the European Medicines Agency (EMA) assessment report on *P. anisum* (EMA, 2012), the use of its EO is considered relatively safe. The British Herbal Pharmacopoeia [86] recommends a posology of 0.05–0.2 mL three times per day for the treatment of mild gastrointestinal complaints and as an expectorant, while the EO dosage per day recommended by the German Commission E is 0.3 g (0.4 mL) [87]. However, since the EO contains methyl chavicol and *trans*-anethole, for which a clear toxicological profile has not been established, the use in sensitive groups such as children, pregnant, and breastfeeding women should be reduced or avoided (EMA, 2012).

3. Insecticidal and acaricidal activity

Aniseed oil and its principal constituents have been extensively studied for their toxicity against agricultural pests, stored-product pests, and vectors [Tables 3-10]. In general, the insecticidal and acaricidal effects of plant EOs, along with their primary constituents, are substantially influenced by their chemical composition [88–90]. Depending on the dose, EOs can either attract or repel insects, or they might serve as a toxin [91]. Because EOs are a complex mixture with numerous constituents, their activity cannot be simplified to a single mechanism of action. In insects, for example, *P. anisum* EO and its primary component, *trans*-anethole, can impair protein activity and inhibit key enzymes [92,93]. *Trans*-anethole can also act as acetylcholinesterase (AChE) inhibitor with systemic effects, neutralizing insect defence mechanisms in the midgut [94,95]. Monoterpenoids, in general, act as AChE inhibitors, but only at large dosages, and their inhibitory impact is reversible [91]. Several of aniseed oil main constituents (e.g., *trans*-anethole and limonene) can be extracted from other plants, such as *Illicium verum, Clausena austroindica, Croton anisatum, Foeniculum vulgare,* or *Zanthoxylum limonella* [96–103]. In the following paragraphs, we reviewed the studies that investigated the efficacy of *P. anisum* EO and its main constituents against veterinary, medical, stored product, and agricultural pests [Tables 3–8]. In addition, assays using *P. anisum* EO-based micro- and nano-emulsions have been reported as well as possible side-effects of aniseed EO and its major compounds on non-target species [Tables 9 and 10].

Table 6
Main components of aniseed oil insecticidal and acaricidal activity evaluated against immature and adult stages of stored product pests. In addition to the mortality rates, the mode of actions is reported; n.a. = not available data.

Compound	Order	Family	Targeted species	Stage	Mode of action	Mortality rates or LC/LD	Notes	References
Estragol Linalool D-Limonene	Coleoptera	Laemophloeidae	*Cryptolestes ferrugineus*	adults	Fumigation	† <2.00% † < 2.00% † < 2.00% LC_{50} = 11.56 μL/mL LC_{90} = 24.11 μL/mL	Extracted from *Illicium verum*	[101]
trans-Anethole	Coleoptera	Curculionidae	*Sitophilus oryzae*	adults	Contact	LC_{50} = 2543.20 μL/L LC_{90} = 4616.22 μL/L	Extracted from *Clausena austroindica*	[102]
					Fumigation	n.a.	LT50 = 13.5 h – dose 11.6 mg/L air LT99 = 61.7 h – dose 11.6 mg/L air	[122]
					Fumigation	LC_{50} = 76.98 μL/L LC_{90} = 125.39 μL/L	Extracted from *Clausena austroindica*	[102]
	Coleoptera	Tenebrionidae	*Tribolium castaneum*	adults	Contact	LC_{50} = 2050.84 μL/L LC_{90} = 2085.05 μL/L	Extracted from *Clausena austroindica*	[102]
					Fumigation	LC_{50} = 29.10 μL/L LC_{90} = 57.31 μL/L	Extracted from *Clausena austroindica*	[102]
	Coleoptera	Tenebrionidae	*Tribolium confusum*	adults	Fumigation	LT_{50} = 10.8 h – dose 11.6 mg/L air LT_{99} = 875.0 h – dose 11.6 mg/L air		[122]
				eggs	Fumigation	LT_{50} = 2.8 h – dose 11.6 mg/L air LT_{99} = 218.8 h – dose 11.6 mg/L air		[122]
	Lepidoptera	Pyralidae	*Ephestia kuehniella*	eggs	Fumigation	LT_{50} = 1.1 h LT_{99} = 117.5 h	dose 11.6 mg/L air	[122]

LC = lethal concentration; LD = lethal dose; LT = lethal time; KT = median lethal time; *trans*-anethole, (*E*)-anethole, anethole and anise camphor are synonyms. Methyl chavicol and estragole are synonyms. n.d. = not detected.

3.1. Arthropods of medical and veterinary interest

A number of arthropods play a crucial role in the transmission of parasites and pathogens from a vertebrate species to another, including humans, livestock, pets, and wildlife [104]. Vector-disease arthropods, especially mosquitoes, have been one of the primary focus of studies on the insecticidal effects of aniseed oil and its major compounds (Tables 3 and 4). *P. anisum* EO has been tested on different mosquito species (Diptera: Culicidae), which represent a major treat to millions of people globally since they act as vectors of many diseases such as malaria, Zika, chikungunya, dengue, and yellow fever [105–108]. Research is mainly focused on the insecticidal activity on mosquito larval stages since larval management is a critical part of a successful mosquito control program [109]. So far, all the experiments conducted on mosquito larvae involving EOs followed the standard procedures established by WHO (1996), with slight modifications. Aniseed oil was very effective against 4th instar larvae of *Culex quinquefasciatus* Say, *Aedes aegypti* L. and *Anopheles stephensi* Liston, though *A. aegypti* and *A. stephensi* larvae were more susceptible than *C. quinquefaciatus* ones (LD_{95} = 115.7 ± 3.3 μg/mL, LD_{95} = 115.7 ± 2.6 μg/mL, LD_{95} = 149.7 ± 1.3 μg/mL, respectively) [110]. Even at low concentration, *P. anisum* EO exerts a relevant toxic effect on *A. aegypti* 3rd instar larvae (LC_{90} = 0.043 ppm after 24 h) [111], and it has been proved to have an ovideterrent effect on females and to cause morphological aberrations at pupal stage [112]. According to Benelli et al. [46], aniseed oil had LC_{90} values of less than 100 ppm against *C. quinquefasciatus* 3rd instar larvae, which is typically sufficient for developing botanical larvicides [113]. In addition, the mosquitocidal activity of aniseed oil and *trans*-anethole on mosquito larvae can be enhanced by combing *P. anisum* EO with *Trachyspermum ammi* (L.) Sprague and *Smyrnium olusatrum* L. (Apiaceae) EOs [88]. Moreover, *trans*-anethole was able to create a synergistic effect with other compounds (e.g., γ-terpinene, eugenol, α-pinene, and carvacol) against *C. quinquefasciatus* larvae [113–115] (Table 4). *trans*-Anethole was also highly effective toward *Blattella germanica* L. (Blattodea: Blattellidae) in contact toxicity assays [116] and against the West Nile vector *Culex pipiens* L. [117].

3.2. Stored product pests

In commodities, aniseed oil showed high effectiveness against many Coleoptera species, making it a promising candidate to protect stored grain within integrated pest management (IPM) programmes (Table 5). The insecticidal activity of aniseed oil was mainly determined via fumigation assays, followed by contact and topical assays [118]. However, the effectiveness of the EO is closely linked to the stage of development of the pest on which it is tested. In general, for stored product pests, eggs, and pupae represent a major challenge since they may be less affected by chemicals than the active stages [119]. For instance, eggs of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) are more tolerant to *P. anisum* EO than the adults [120,121]. However, in fumigant experiments with *trans*-anethole, the results were diametrically opposed, with the eggs of *T. confusum* being more sensitive than adults [122].

Recent studies not only investigated *P. anisum* EO and *trans*-anethole toxic effect but also observed their action at an enzymatic level. For instance, *trans*-anethole was responsible for the decline of AChE activity in *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (LC_{50} = 5.02 mg/L air, after 24 h) [123], for the interaction with the detoxicant system of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) [92,95] and an increased activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) in *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), enzymes that play a pivotal role in the elimination of reactive oxygen species (ROS) products in cells [124].

3.3. Agricultural pests

The efficacy of *P. anisum* EO was evaluated on several species of agricultural interest (Tables 7 and 8). On adults of the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), *P. anisum* EO showed promising results in acute toxicity assays when tested by contact (spraying) [46], while *M. persicae* nymphs were more resistant compared to nymphs of *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) in fumigation assays [125]. Indeed, the lowest dose (i.e., 0.25 μL/L of air) caused the 87.5% of

Table 7
P. anisum EO activity evaluated against immature and adult stages of agricultural pests. In addition to the mortality rates, the mode of action and the percentage of main compounds are reported; n.a. = not available data.

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
Coleoptera	Chrysomelidae	*Leptinotarsa decemlineata*	2nd instar larvae	Contact	Commercial EO	LC_{50} = 1.76% (v/v) (24 h) LC_{50} = 0.45% (v/v) (120 h) LC_{90} = 8.29% (v/v) (24 h) LC_{90} = 1.01% (v/v) (120 h)		[203]
				Contact	Commercial EO	LC_{50} = 1.7 ppm (24 h) LC_{90} = 9.5 ppm (24 h)		[139]
				Contact (topical)	Commercial EO	LC_{50} > 20.0 ppm (24 h) LC_{90} > 20.0 ppm (24 h)		[139]
				Ingestion	Commercial EO	LC_{50} = 1.17 µL/larva (24 h), LC_{50} = 0.43 µL/larva (120 h) LC_{90} = 2.86 µL/larva (24 h), LC_{90} = 1.78 µL/larva (120 h)		[139]
Coleoptera	Bostrichidae	*Ips typographus*	adults	Contact	Anethole 88.6%; estragol 4.4%; linalool 1.4%; camphene 0.8%; α-pinene 0.7%; α-phellandrene 0.5%; isocaryophyllene 0.3%; 4-terpineol 0.2%	LD_{50} = 0.117 µL/cm^2 (72 h) LD_{50} = 0.053 µL/cm^2 (96 h) LD_{90} = 0.645 µL/cm^2 (72 h) LD_{90} = 0.139 µL/cm^2 (96 h)		[204]
Coleoptera	Bostrichidae	*Rhyzopertha dominica*	adults	Fumigation	n.a.	† 75% - dose 15 µL/L (24 h) † 62% - dose 10 µL/L (24 h) † 10% - dose 5 µL/L (24 h)		[195]
Hemiptera	Aphididae	*Acyrthosiphon pisum*	nymphs	Fumigation	n.a.	100% † - dose 2 µL/L air, 1 µL/L air, and 0.5 µL/L 87.28% † - dose 0.25 µL/L		[125]
Hemiptera	Aphididae	*Aphis gossypii*	adults	Vapor	See Saraç & Tunc 1995	† 96.7% (24 h)	dose 2.00 µL/L	[205]
Hemiptera	Aphididae	*Brevicoryne brassicae*	adults	Fumigation	Commercial EO	† 26.6% (daily deaths/daily offspring)		[206]
				Spray	n.a.	cumulative † 27% - dose 1% (72 h) cumulative † 43% - dose 10% (72 h)		[207]
			nymphs	Spray	n.a.	cumulative † 17% - dose 1% (72 h) cumulative † 27% - dose 10% (72 h)		[207]
Hemiptera	Aphididae	*Lipaphis pseudobrassicae*	adults	Contact	(E)-Anethole (85%); methyl chavicol (6%)	LC_{50} = 4.6 mg/mL (60 min) LC_{50} = 4.9 mg/mL (30 min) LC_{50} = 6.9 mg/mL (10 min)		[208]
Hemiptera	Aphididae	*Macrosiphum euphorbiae*	2nd/3rd nymphs	Fumigation	*trans*-Anethole (87.3%); estragol (3.91%); linalool (1.86%); limonene (1.14%); folliculin (1.07%); linalyle benzoate (0.66%); α-pinene (0.58%); anisaldehyde (0.52%)	LC_{50} = 6.6 µL/L (24 h)		[209]
Hemiptera	Aphididae	*Myzus persicae*	n.a.	Contact		LC_{50} = 0.03 µL/mL		[210]
			adults	Spray	*trans*-Anethole (93.0%); methyl cavicol (15.0%); *p*–anisaldehyde (1.7%); γ-himachalene (1.5%)	LC_{50} = 4.3 mL/L (48 h) LC_{90} = 9.5 mL/L (48 h)		[46]
			nymphs	Fumigation	n.a.	† 95% - dose 2 µL/L air † 80% - dose 1 µL/L air		[125]
Hemiptera	Aphididae	*Nasonovia ribisnigri*	adults	Contact (growth chamber)	Commercial EO	EO 0.4%, efficacy of 53.8 after 1 d, 64.8 after 2 d, 70.2 (3 days), 68.2 after 6 d		[137]
				Contact (greenhouse)	Commercial EO	EO 0.4%, efficacy 17.4–31.8 after 1 d, 27.4–47.1 after 2 d, 40.1–47.5 (3 days),		[137]

(continued on next page)

Table 7 (*continued*)

Order	Family	Target species	Stage	Mode of action	Main constituents	Mortality rates or LC/LD		Notes	References
						25.0–44.1 after 6 d; with EO 0.2%, efficacy 16.3 after 1 d, 22.7 after 2 d, 15.0 (3 days), 25.9 after 6 d;			
				Contact (open field)	Commercial EO	EO 0.2%, efficacy 62.6 after 1 d, 51.8 after 2 d, 17.1 after 1w EO 0.3%, efficacy 47.6 after 1 d, 52.0 after 2 d, −18.3 after 1w			[137]
Diptera	Tephritidae	*Bactrocera oleae*	adults	Ingestion	*trans*-Anethole (98.3%); methyl chavicol (0.8%); (E)-pseudoisoeugenyl 2-methylbutyrate (0.6)	LC_{50} = 771 ppm LC_{90} = 1981 ppm		† Checked daily for 4 days	[211]
Diptera	Sciaridae	*Lycoriella ingenua*	larvae	Fumigation	Commercial EO	†100% - dose 25 μL/L and 10 μL/L † 96.6% - dose 5 μL/L and 2.5 μL/L † 93.3% - dose 1.25 μL/L			[212]
Lepidoptera	Noctuidae	*Spodoptera littoralis*	3rd instar larvae	Contact	*trans*-Anethole (93.0%); methyl chavicol (15.0%); *p*–anisaldehyde (1.7%); γ-himachalene (1.5%)	LC_{50} = 57.3 μg/larva (24 h) LC_{90} = 87.8 μg/larva (24 h)			[46]
Lepidoptera	Noctuidae		4th instar larvae	Ingestion	n.a.	LC_{50} = 38.5 ppm (24 h) LC_{95} = 78.0 ppm (24 h)			[213]
			eggs	Fumigation	n.a.	at 100 ppm, † 78.6% after 120 h at 100 ppm, † 78.6% after 120 h			[213]
Trombidiformes	Tetranychidae	*Tetranychus cinnabarinus*	adults	Vapor	n.a.	LT_{50} = 27.5 LT_{50} = 20.9 LT_{50} = 14.0 LT_{50} = 17.4	LT_{90} = 182.0 LT_{90} = 61.7 LT_{90} = 51.3 LT_{90} = 63.1	Dose 0.25 μL/L Dose 0.50 μL/L Dose 1.00 μL/L Dose 2.00 μL/L	[205]
Trombidiformes	Tetranychidae	*Tetranychus urticae*	adults	Contact	*trans*-Anethole (53.23%); estragole (13.52%); caryophyllene (1.26%)	LC_{50} = 22.32 μL/l (24 h) LC_{50} = 21.73 μL/l (48 h) LC_{50} = 20.94 μL/l (72 h)	LC_{90} = 43.98 μL/l (24 h) LC_{90} = 39.99 μL/l (48 h) LC_{90} = 35.80 μL/l (72 h)		[127]

LC = lethal concentration; LD = lethal dose; LT = lethal time; KT = median lethal time; *trans*-anethole, (*E*)-anethole, anethole and anise camphor are synonyms. Methyl chavicol and estragole are synonyms. n.d. = not detected.

mortality on *A. pisum*, but no mortality was observed for *M. persicae* [125]. A possible explanation for this discrepancy lies in the wide host-range of *M. persicae*. Indeed, generalist phytophagous insects that feed on a wide variety of plants have been discovered to have higher amounts of cytochrome P450 monooxygenase activity in their gut, which allows them to detoxify plant defensive compounds more efficiently [126]. As in the case of stored product pests, studies have been conducted for some agricultural pests to assess the effect of EOs at a physiological level. Aniseed oil at 40 μL/L could fully control the two-spotted spider mite, *Tetranychus urticae* C.L. Koch (Trombidiformes), causing 96% of mortality after 72 h [127] and affecting the functioning of AChE and protease. Investigating the neurotoxic effect of *trans*-anethole on *Hypantria cunea* (Drury) larvae (Lepidoptera: Arctiidae), Pour et al. [128] noted that this compound strongly suppressed the AChE activity, exhibiting neurotoxic effects. Lastly, *trans*-anethole exhibits a synergistic effect when used in combination with thymol and α-terpineol against moths of agricultural interest, such as *Spodoptera litura* (Fabricius), *Spodoptera littoralis* (Boisduval), *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and *Chilo partellus* (C. Swinhoe) (Lepidoptera: Pyralidae) [129–131].

3.4. Micro- and nano-emulsions

Some of the limitations to the use of EO as biopesticides are their low chemical stability, limited persistence in the environment, and the poor hydrophilicity [92,132]. A good strategy to overcome these drawbacks is represented by the development of micro- (MEs) and nanoemulsion (NEs) EO-based formulations [92,132]. Aniseed EO-based nanotechnologies proved to be effective on different pest species and developmental stages, even at low concentrations, compared to conventional EO formulations. For instance, in a study carried out by Draz et al. [133], aniseed NE formulations were significantly more toxic (1.50 and 1.41 times) against *T. castaneum* and *S. oryzae* than the conventional EO, without affecting the wheat germination rate. Corn derived zein-based nanocapsules loaded with aniseed EO have been successfully tested against 3rd instar larvae of *C. quinquefasciatus*, showing to be effective at lower doses than aniseed EO alone (*P. anisum* EO: LC_{50} = 25.9 μL/L and LC_{90} = 31.9 μL/L; *P. anisum* EO microemulsion: LC_{50} = 2.39 μL/L and LC_{90} = 4.13 μL/L) [134].

Table 8
Main components of aniseed oil insecticidal and acaricidal activity evaluated against immature and adult stages of agricultural pests. In addition to the mortality rates, the mode of actions is reported; n.a. = not available data.

Compound	Order	Family	Targeted species	Stage	Mode of action	Mortality rates or LC/LD	Notes	References
estragol	Diptera	Tephritidae	*Bactrocera cucurbitae*	adults	Fumigation	LT_{90} = 15 min		[214]
	Diptera	Tephritidae	*Bactrocera dorsalis*	adults	Fumigation	LT_{90} = 8 min		[214]
	Diptera	Tephritidae	*Ceratitis capitata*	adults	Fumigation	LT_{90} = 15 min		[214]
trans-anethole	Hemiptera	Aphididae	*Myzus persicae*	adults	Fumigation	LC_{50} = 1.292 mL/L (SD) LC_{50} = 0.415 mL/L (OEE) LC_{50} = 0.336 mL/L free vapors LC_{90} = 3.383 mL/L (SD) LC_{90} = 0.780 mL/L (OEE) LC_{90} = 1.043 mL/L free vapors		[215]
	Hemiptera	Aphididae	*Nasonovia ribisnigri*	adults	Contact (growth chamber)	Dose 0.4% efficacy 51.9% (1 day), 55.1% (2 days), 59.4% (3 days), 23.1% (6 days)		[137]
					Contact (greenhouse)	Dose 0.4% efficacy 14.7–30.0% (1 day), 37.6–42.2% (2 days), 40.7–41.8% (3 days), 21.6–41.9% (6 days) Dose 0.2% efficacy 18.1% (1 day), 20.7% (2 days), 16.5% (3 days), 32.2% (6 days)		
					Contact (open field)	Dose 0.3% efficacy 49.0% (1 day), 50.5% (2 days), 38.4% (1 week) Dose 0.2% efficacy 44.2% (1 day), 39.8% (2 days), −8.8% (1 week)		
	Diptera	Tephritidae	*Bactrocera cucurbitae*	adults	Fumigation	LT_{90} = 29 min		[214]
	Diptera	Tephritidae	*Bactrocera dorsalis*	adults	Fumigation	LT_{90} = 26 min		[214]
	Diptera	Tephritidae	*Ceratitis capitata*	adults	Fumigation	LT_{90} = 17 min		[214]
	Diptera	Drosophilidae	*Drosophila suzukii*	adults	Contact	LD_{50} = 1.75 mg/L ♂ (24 h) LD_{50} = 3.0 mg/L ♀ (24 h)	Extracted from *Illicium verum* and *Croton anisatum*	[98]
	Diptera	Sciaridae	*Lycoriella ingenua*	larvae	Fumigation	LC_{50} = 0.20 μL/L (24 h)		[212]
	Lepidoptera	Crambidae	*Chilo partellus*	3rd instar larvae	Contact (topical)	LD50 = 409.7 μg/larva		[216]
	Lepidoptera	Pyralidae	*Ephestia kuehniella*		Fumigation	LT_{50} = 40.7 at 2.9 mg/L LT_{50} = 2.5 at 5.8 mg/L LT_{50} = 1.1 and at 11.6 mg/L LT_{99} = 117.5 at 11.6 mg/L		[122]
	Lepidoptera	Erebidae	*Hyphantria cunea*	4th instar larvae	Ingestion	LC_{50} = 1.41 μL/mL LC_{90} = 7.20 μL/mL	At LC_{50} showed 87% feeding deterrence	[128]
	Lepidoptera	Noctuidae	*Helicoverpa armigera*	3rd instar larvae	Contact (topical)	LD_{50} = 378.6 μg/larva		[216]
	Lepidoptera	Noctuidae	*Spodoptera litura*	4th instar larvae	Contact (topical)	LD_{50} = 65.5 μg/larva LD_{90} = 98.8 μg/larva		[129]
				3rd instar larvae	Contact (topical)	LD_{50} = 64.3 μg/larva		[216]

LC = lethal concentration; LD = lethal dose; LT = lethal time; KT = median lethal time; *trans*-anethole, (*E*)-anethole, anethole and anise camphor are synonyms. Methyl chavicol and estragole are synonyms. n.d. = not detected.

Thanks to their structure and composition, ME and NE droplets may show increased dispersion and facilitate the release of EO active compounds in the environment, and also reduce the occurrence of undesirable phytotoxic effects on treated plants [135,136]. *P. anisum* NE at 0.4% (v/v) reduced the colony development of the aphid *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae) without causing phytotoxicity on sprayed lettuces in growth chambers, greenhouses, and open-field experiments [137]. Sometimes, nanoformulations may not be better in terms of increased toxicity but, due to their chemical–physical properties, they may enhance other effects, like repellence and deterrence [134]. Olives treated with aniseed NE at 7.5% showed a significant reduction in oviposition by the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), although the formulation did not exert a relevant contact toxicity [138]. In trials conducted on 2nd instar larvae of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), Skuhrovec et al. [139] found that conventional aniseed EO is slightly more efficient against this beetle compared to NE formulations when applied topically, by contact or orally, but the NE formulation exhibits more than 20 times the persistency and almost twice the antifeedant activity of the conventional EO.

As for classical EO formulations, NEs may exhibit variable effects depending on the developmental stage on which they are tested. Kavallieratos et al. [132] showed that aniseed EO-based NE at 4% w/w has low mortality rate on the adults of *T. castaneum* and *T. confusum* (30.1% and 13.3% at 1000 ppm after 7 days of exposure, respectively) while exerting a moderate to strong effect on their larval stages (mortality of 81.4% after

Table 9
Micro- and nano-emulsion of aniseed oil and its major component toward medical, veterinary, stored product and agricultural pests. In addition to the mortality rates, the mode of actions is reported; n.a. = not available data.

Order	Family	Target species	Stage	Formulation		Mode of action	Mortality rates or LC/LD	notes	references
Diptera	Culicidae	*Culex quinquefasciatus*	3rd instar larvae	Loaded-zein NC	n.a.	Aqueous solution	LC_{50} = 40.6 μL/L (24 h) LC_{90} = 66.4 μL/L (24 h)		[134]
				ME	n.a.	Aqueous solution	LC_{50} = 2.39 μL/L (24 h) LC_{90} = 4.13 μL/L (24 h)		[134]
				ME 1.5%	n.a.	Contact	LC_{50} = 2.39 mL/L (24 h) LC_{90} = 4.13 mL/L (24 h)		[56]
				ME 1.125	n.a.	Contact	LC_{50} = 4.01 mL/L (24 h) LC_{90} = 6.48 mL/L (24 h)		[56]
Sarcoptiformes	Acaridae	*Acarus siro*	adults	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	Treated wheat	† 18.6% - dose 500 ppm (7 days) † 38.1% - dose 1000 ppm (7 days)	NE (3% *T. ammi* EO - *P. anisum* EO) was also investigated	[132]
			nymphs	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	Treated wheat	† 18.6% - dose 4% (7 days)	NE (3% *T. ammi* EO - *P. anisum* EO) was also investigated	[132]
Coleoptera	Curculionidae	*Sitophilus oryzae*	adults	NE	5% (o/w) *P. anisum* EO + 10% TWEEN80	?	LC_{50} = 3858.88 mg/L		[133]
Coleoptera	Tenebrionidae	*Tenebrio molitor*	adults	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	Treated wheat	† 17.5% - dose 4% (7 days)		[132]
			larvae	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	Treated wheat	† 2.2% - dose 4% (7 days)		[132]
Coleoptera	Tenebrionidae	*Tribolium castaneum*	adults	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	Treated wheat	† 0% (4–16 h) † 0% (1–2 days) † 2.2% (3–4 days) † 5.6% (5 days) † 6.8% (6 days) † 8.2% (7 days)	Dose 4%	[132]
				NE	5% *P. anisum* EO + 10% TWEEN80	n.a.	LC_{50} = 4985.1 mg/L	Essential oil in water (O/W) nano-emulsions	[133]
				NE 14%	*P. anisum* EO + ethanol 3% + Tween 80 (3%)	Contact+ Ingestion	LC_{50} = 9.8% (v/v)	Essential oil in water (O/W) nano-emulsions	[92]
				NE 14%	*P. anisum* EO + ethanol 3% + Tween 80 (3%)	Contact+ Ingestion	LC_{50} = 9.8% (v/v)		[93]
			larvae	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	Treated wheat	† 1.1% (4 h) † 7.8% (8 h) † 10% (16 h) † 21.1% (1 days) † 38.9% (2 days) † 57.8% (3 days) † 68.6% (4 days) † 76.3% (5 days) † 78.4% (6 days) † 81.4% (7 days)	Dose 4%	[132]
Coleoptera	Tenebrionidae	*Tribolium confusum*	adults	NE	n.a.	aerosol	LC_{50} = 2.561 mg/L (24 h) LC_{50} = 2.099 mg/L (1 week)	RC_{50} = 0.042 mg (24 h) RC_{50} = 0.033 mg (48 h)	[217]
				NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	treated wheat	† 2.2% (7 days)	Dose 4%	[132]
			larvae	NE	4% (w/w) *P. anisum* EO + 4%	treated wheat	† 3.3% (1 day) † 21.1% (5 days)		[132]

(continued on next page)

Table 9 (*continued*)

Order	Family	Target species	Stage	Formulation		Mode of action	Mortality rates or LC/LD		notes	references
					(w/w) polysorbate 80		† 6.7% (2 days) † 12.2% (3 days) † 18.9% (4 days)	† 25.6% (6 days) † 27% (7 days)		
Coleoptera	Dermestidae	*Trogoderma granarium*	adults	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	treated wheat	† 1.1% (16 h) † 6.7% (1 day) † 16.7% (2 days) † 28.9% (3 days)	† 40.6% (4 days) † 47.5% (5 days) † 58% (6 days) † 64.8% (7 days)	Dose 4%	[132]
			larvae	NE	4% (w/w) *P. anisum* EO + 4% (w/w) polysorbate 80	treated wheat	† 1.2% (6 days) † 2.5% (7 days)			[132]
Coleoptera	Chrysomelidae	*Leptinotarsa decemlineata*	2nd instar larvae	MC	10% *P. anisum* + Tween 80	contact	LC_{50} = 3.1 ppm (24 h) LC_{90} = 14.3 ppm (24 h)			[139]
				MC		contact (topical)	LC_{50} > 20.0 ppm (24 h)			[139]
				MC		ingestion	LC_{50} = 0.47 μL/larva (24 h) LC_{50} = 0.09 μL/larva (120 h) LC_{90} = 1.46 μL/larva (24 h) LC_{90} = 0.42 μL/larva (120 h)			[139]
Diptera	Tephritidae	*Bactrocera oleae*	adults	NE	15% *P. anisum* EO + 5% Tween 80 + 80% H_2O	contact	no † at under 3.75% dose. at 5.00% and 7.50% dose, † 1.67%;		Essential oil in water (O/W) nano-emulsions	[138]

LC = lethal concentration; LD = lethal dose; LT = lethal time. n.d. = not detected.

7 days of exposure at 500 ppm or 98.9% after 5 days at 1000 ppm on *T. castaneum* larvae; 63.1% after 7 days at 1000 ppm on *T. confusum* larvae). Against particularly resistant pests, EO-based nanoformulations may be useful to overcome the limited efficacy of classical natural or synthetic insecticides. Aniseed and ajwain, *T. ammi*, NE formulations showed low to moderate mortality when tested on adults of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) separately, but a stronger effect emerged when combined in a 3% w/w *P. anisum* + 3% w/w *T. ammi* nanoemulsion, indicating that certain combinations of EOs in NEs may exhibit additive effects on certain species or developmental stages [132]. Similar results have been obtained by Pavela et al. [56], testing highly stable MEs loaded with *P. anisum* EO in combination with two other EOs extracted from Apiaceae, i.e., *T. ammi* and *Crithmum maritimum* L. All the tested MEs caused acute toxicity to *C. quinquefasciatus* 3rd instar larvae (LC_{50} values ranging 1.45–4.01 mL/L) and a significant synergistic effect emerged in MEs loaded with *P. anisum* and *T. ammi* EOs [56]. In both cases, a possible explanation may be related to the combined action of the two major compounds in the mixture, i.e., *trans*-anethole (from *P. anisum*) and thymol (from *T. ammi*), the first one acting by neutralizing the detoxification system of the insect (cytochrome P450, glutathione-S-transferases and esterases) and second one being able to inhibit the acetylcholinesterase and interact with octopamine receptors modulating GABA channels [56,88,94,124,140].

3.5. Effects of aniseed EO on non-target species

Table 7 summarizes the current knowledge about the toxicity of aniseed EOs and their main chemical constituents against different non-target vertebrate and invertebrate species. The available data depict a limited though complex scenario: even though studies investigating the insecticidal and acaricidal potential of EOs became more and more widespread, most of them tested the efficacy of a single or more EOs on one or more target organisms, forgetting to extend effect assessments to non-target organisms as well, a critical point in the procedure for the authorisation of a biopesticide [141–143].

In the case of *P. anisum*, Pavela [114] observed that both the EO and its main compound, *trans*-anethole, were toxic for *Daphnia magna* Straus (Cladocera: Daphniidae) and negatively influenced its fertility at high concentrations (35–50 μL/mL) and long exposure (48 h), but these effects were extremely reduced at lower concentrations (20 μL/mL) and short exposures (6 h). Similarly, Sánchez-Gómez et al. [134] found that *P. anisum* EO-loaded nanocapsules had a negative impact on *D. magna* adults after a 48-h exposure but argued that this effect significantly decreased with exposures shorter than 24 h. Testing aniseed EO in laboratory assays, Benelli et al. [46] found scarce toxicity on 3rd instar larvae of the multi-coloured Asian ladybug *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), a useful predator for the control of aphid populations (maximum mortality 16.3% at 5.5 mL/L), while no mortality was observed on adults. However, a particular attention must be paid to how these formulations are used, as high doses and long exposure may have sub-lethal effects, such as reduced fertility or behavioural modifications, in non-target organisms [131,144]. For instance, aniseed EO has been shown to have low toxicity (i.e., high LCs) on *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), a voracious predatory mirid largely employed in the biological control of pests in the Mediterranean area [145], even if higher concentrations of the oil may result in decrease of the insect's fertility and orientation ability [144]. Aniseed oil showed to drastically reduce the emergence rate of wasps from the parasitized eggs of *E. kuehniella,* by the parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae), while its impact on parasitoid

Table 10
Aniseed EO, its nanoformulation and major compounds against non-target organisms. In addition to the mortality rates, the mode of actions is reported; n.a. = not available data.

Order	Family	Target species	Stage	Tested product	Mode of action	Main constituents	Mortality rates or LC/LD	Notes	References
Cladocera	Daphniidae	*Daphnia magna*	adults	EO	Aqueous solution	*trans*-anethole (81.33%); γ-himachalene (12.32%); α-himachalene (0.96%); linalool (0.85%); δ-elemene (0.54%)	LC_{50} = 31 μL/L		[114]
				trans-anethole	Aqueous solution	–	LC_{50} = 29 μL/L		[114]
				P. anisum loaded-zein NC	Aqueous solution	*trans*-anethole (93.0%)	† 29.3% - dose 30 μL/L (48 h)		[134]
Haplotaxida	Lumbricidae	*Eisenia fetida*	adults	NC	Soil mixture	*trans*-anethole (93.0%)	† 2.5% - dose 30 μL/kg (7 days)		[134]
				EO	Soil mixture	*trans*-anethole (93.0%); *p*-anisaldehyde (1.7%); γ-himachalene (1.5%); methyl chavicol (1.5%); (E)-pseudoisoeugenyl 2-methylbutyrate (1.1%); α-zingiberene (0.3%)	† 0.0% - dose 30 μL/kg (7 days)		[46]
Hemiptera	Miridae	*Nesodiocoris tenuis*	adults	NE	Topical contact	*trans*-anethole (86.54%) (Campolo et al., 2020)	LC_{30} = 4.547 mg/mL	Effect on fertility and orientation	[144]
Coleoptera	Coccinellidae	*Harmonia axiridis*	adults	EO	Spray	*trans*-anethole (93.0%); *p*-anisaldehyde (1.7%); γ-himachalene (1.5%); methyl chavicol (1.5%); (E)-pseudoisoeugenyl 2-methylbutyrate (1.1%); α-zingiberene (0.3%)	† 0% - dose 5.5 mL/L		[46]
			3rd instar larvae		Spray	*trans*-anethole (93.0%); *p*-anisaldehyde (1.7%); γ-himachalene (1.5%); methyl chavicol (1.5%); (E)-pseudoisoeugenyl 2-methylbutyrate (1.1%); α-zingiberene (0.3%)	† 16.3% - dose 5.5 mL/L		[46]
Hymenoptera	Trichogrammatidae	*Trichogramma evanescens*	parasitized eggs	EO	Fumigation	commercial EO		Anise EO are highly toxic for parasitoid development	[146]

LC = lethal concentration; LD = lethal dose; LT = lethal time; KT = median lethal time; *trans*-anethole, (*E*)-anethole, anethole and anise camphor are synonyms. Methyl chavicol and estragole are synonyms. n.d. = not detected.

behaviour (repellence vs. attraction) may vary according to the selected strain of the wasp, a further aspect to be considered when selecting the EO [146].

4. Conclusion and future challenges

Due to the negative effects of massive pesticide use on agro-ecosystem biodiversity and human health [147], as well as the withdrawal of several recently revealed harmful products, the use of alternative plant-based pesticides, such as EOs, should be encouraged. In this perspective, *P. anisum* EO proved its efficacy against many arthropods by exerting neurotoxic effects, via GABA receptors, octopamine synapses, and the inhibition of AChE [92]. One of the most promising areas of application of *P. anisum* EO is against arthropods of medical and veterinary importance, where it was successfully tested against several mosquito species [46,105,110,112–115]. At low doses and short time of exposure, either the EO or the nanoformulations showed low toxicity toward aquatic non-target species, while maintaining their effectiveness toward vector larval stages [114,134]. In addition, dilution in water may facilitate the delivery of the substance to target organisms [105–110,112–115]. However, to date, most studies concerning EOs, including *P. anisum*, are based on standardized laboratory bioassays, while field studies are still uncommon. The possibility of discrepant results between laboratory assays and field tests must be considered. There are still several limits to be overcome in the application of EO-based bioinsecticides in IPM programs [91,94]. Further studies are needed to carefully evaluate the effects of EOs on organisms according to their developmental stage or genetic strain, as well as their toxic or sublethal effects on non-target species and the post-application impacts.

Because of its use in food, beverages, cosmetics, and fragrances, aniseed cultivation all over the world, particularly in the Mediterranean and Western Asian countries, ensures the plant biomass required to extract the EO and its use in the agrochemical industry even without further crop system implementation. Its low cost (1 kg of EO costs 7–50 euro/kg depending on geographic origin and growth system) is another advantage for using this EO to make biopesticides [8,148]. It is essential to conduct additional research on enhancing the intensification of farming technology to increase yields. A high biological yield can be achieved by a proper agronomic practice and a profitable selection variety or different stages of plant maturity [149,150], or by changing the EOs isolation technology [151]. Furthermore, the constancy of the aniseed chemical profile documented in the literature, resulting in an almost 'monocomponent' EO, eliminates the possibility of insecticidal efficacy fluctuation. Botanical compound-based micro- and/or nano-systems may represent a stumbling block in pest management programs. The encapsulation of active compounds can partially address some of the issues in EO application, such as thermolability and photolability, increasing their overall efficiency. Among available nanosystems, MEs and NEs are the most suited for EOs given their high lipophilicity [152]. Moreover, through the creation of binary or tertiary mixtures of different EOs, micro- and nanocapsules can allow the arise of synergic effects related to a conjugate action of their major constituent, although some of the mechanisms underlying these interactions remain to be clarified. Lastly, a cost reduction of the overall process should be pursued.

Declaration of interests

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. Giovanni Benelli is an Editorial Board Member of Agriculture Communications, but was not involved in the peer-review process of this article.

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