Artemisia Genus as Biopesticides

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The Artemisia L. genus includes over 500 species with worldwide distribution and diverse chemical composition. Many secondary metabolites of this genus are known for their antimicrobial, insecticidal, parasiticidal, and phytotoxic properties, which recommend them as possible biological control agents against plant pests. Given the negative impact of synthetic pesticides on human health and on the environment, Artemisia-derived biopesticides and their nanoformulations emerge as promising ecofriendly alternatives to pest management.

antifungal antibacterial insecticidal nematicidal phytotoxic

1. Introduction

The *Artemisia* L. genus contains over 500 species, herbaceous plants and shrubs, widespread in the northern hemisphere, in Asia, Europe, and North America. *Artemisia* species are found in various ecosystems, ranging from arid regions to wetland at sea level as well as in the mountains. The largest number of species are located in the steppes of Asia [1]. Common names of *Artemisia* species are wormwood, mugwort, and sagebrush. Due to their biological and chemical diversity, *Artemisia* species have numerous applications in the treatment of plant and human diseases, in cosmetic and pharmaceutical industry. In addition, various *Artemisia* species are used all over the world as foods, spices, condiments, and beverages [2]. Many important medicinal plants belong to this genus and exert a range of therapeutic actions: antibacterial, antifungal, antiviral, antiprotozoal, anthelmintic, anti-inflammatory, anti-ulcer, appetite stimulating, hepatoprotective, antispasmodic, bronchodilator, hypolipidemic, antihypertensive, analgesic, neuroprotective, neurotrophic, anti-depressant, antioxidant, cytotoxic, antitumor, estrogenic, anti-allergic, immunomodulatory, insecticidal, repellent, and anticonvulsant [3][4][5][6][7][8].

Most *Artemisia* species are aromatic plants that produce volatile oil in the secretory hairs on the aerial organs but also through the secretory ducts in the parenchyma tissues. Essential oils could be used as biocontrol agents based on the antibacterial, antifungal, repellent, insecticidal, nematicidal, and phytotoxic effect of volatile compounds. Moreover, the complex mixture of substances with different mechanisms of action, often having synergistic activity, can be effective in preventing the emergence of resistant strains of phytopathogens [9][10][11][12].

The global use of synthetic pesticides has many disadvantages, such as high cost, danger to non-target organisms, accumulation of pesticide residues in the environment, the emergence of resistant phytopathogenic strains, and negative impact on human health [12]. In contrast, biological pesticides can achieve pest management in an environmentally friendly way and could become safer alternatives for the treatment of crop diseases. Many agents are considered biopesticides, such as viruses, microbes, fungi, entomophagous invertebrates, parasitoids,

predators, and substances produced by living organisms such as bacteria, fungi, plants, algae, animals, etc. Throughout this review, we will use the word “biopesticides” for plant-derived substances or extracts. During evolution, plants developed different mechanisms to defend themselves from predators and diseases by producing substances with bactericidal, fungicidal, insecticidal, nematicidal, or repellent activity. At present, these phytochemicals are explored as biocontrol agents for crops integrated pest management. Plant compounds are cheaper, safer for farmers, less toxic to non-target organisms, and rapidly degraded in the environment [13].

In this context, numerous researchers have identified new potential biopesticides in plants of the *Artemisia* genus. Since most species are fragrant, the vast majority of investigations have focused on the biological actions of volatile oils and compounds. Essential oils contain a variety of volatile molecules such as mono- and sesquiterpenes as well as phenolic-derived aromatic and aliphatic components [1]. The percentage of individual compounds in the essential oil is variable and depends on genetic factors (species, chemotype), plant origin, plant organ, period of harvest or developmental stage, environmental factors (climate, altitude, sun exposure), and cultivation conditions. Qualitative and quantitative differences in the composition of the essential oil can also be caused by drying methods, extraction procedure and time, quantification methods, and conditions of analysis [11]. All these elements could change the chemical composition of an essential oil, leading to changes in activity; thus, standardization is necessary to guarantee the effect, and also for regulatory and marketing purposes. Moreover, plants with desirable pesticide action may give low yields of essential oil, hence the need for new and more efficient extraction methods, which will increase the quantity and quality of extracted oil while reducing the time and cost of extraction [14].

2. *Artemisia* Compounds and Extracts with Pesticide Activity

2.1. Antifungal and Anti-Oomycete Activity

Pathogenic fungi produce almost 30% of crop diseases, threatening the health and food security of a growing human population dependent on substantial agricultural production [15]. Phytopathogenic fungi affect plants during their cultivation or after harvest, causing significant losses in crop plants. In addition, certain fungi (*Aspergillus* spp., *Fusarium* spp., *Alternaria* spp. etc.) produce mycotoxins that endanger the health of consumers through hepatotoxic, nephrotoxic, and carcinogenic effects or even cause death [16]. In an effort to find an ecological solution to this problem, numerous studies have assessed the antifungal effect of *Artemisia* species, focusing especially on volatile oil and compounds. Different methods of evaluation were used in vitro, in planta, or in field conditions, and the results were expressed in various ways: half maximal inhibitory concentration—IC_{50}, minimal inhibitory concentration—MIC, minimum fungicidal concentration—MFC, median effective concentration—ED_{50}, inhibition zone, and percent of inhibition (Table 1).

Table 1. Antifungal activity of *Artemisia* extracts and compounds against phytopathogenic fungi.

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
A. abrotanum fresh aerial parts	essential oil (eucalyptol)	*Sclerotinia sclerotiorum*	MIC = 1200 µL/L	in vitro	[17]
A. absinthium aerial parts	essential oil (cis-epoxyocimene, (−)-cis-chrysanthenol, chrysanthenyl acetate, linalool and β-caryophyllene)	*Botrytis cinerea*	ED_{50} = 0.01–0.07 mg/mL	in vitro	[18]
		Fusarium moniliforme	ED_{50} = 0.24–0.43 mg/mL		
		F. oxysporum	ED_{50} = 0.29–0.40 mg/mL		
		F. solani	ED_{50} = 0.24–0.50 mg/mL		
A. absinthium leaves	aqueous extract (1:1)	*Alternaria alternata*	79.75% inhibition	in vitro	[19]
		Mucor piriformis	73.04% inhibition		
		Penicillium expansum	75.42% inhibition		
A. annua fresh aerial parts	essential oil (artemisia ketone)	*Sclerotinia sclerotiorum*	MIC = 2400 µL/L	in vitro	[17]
A. annua aerial parts	essential oil (artemisia ketone, α-selinene and γ-terpineol)	*Alternaria solani*	EC_{50} = 21.78 mg/mL	in vitro agar diffusion	[20]
			EC_{50} = 14.18 mg/mL	in vitro spore germination	
A. annua leaves	methanol extract (ultrasound-assisted)	*Fusarium oxysporum*	36.94% inhibition	in vitro	[21]
	essential oil (camphor, germacrene D, β-caryophyllene, camphene)	*F. oxysporum*	MIC = 0.22 mg/mL		
		F. solani	MIC = 0.37 mg/mL		
	L-camphor	*F. oxysporum*	MIC = 0.11 mg/mL		

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
		F. solani	MIC = 0.31 mg/mL		
	DL-camphor	*F. oxysporum*	MIC = 0.14 mg/mL		
		F. solani	MIC = 0.16 mg/mL		
	β-caryophyllene	*F. oxysporum*	MIC = 0.13 mg/mL		
		F. solani	MIC = 0.23 mg/mL		
	camphene	*F. oxysporum*	MIC = 0.16 mg/mL		
		F. solani	MIC = 0.22 mg/mL		
	petroleum ether extract	*F. oxysporum*, *F. solani*	27.78% and 25% infection incidence, at 0.25 mg/g and 0.5 mg/g in the culture media, respectively	in vivo on *Panax notoginseng*	
A. annua whole plant	ethanol extract	*Aspergillus flavus*	14 mm inhibition zone at 200 µg/mL	in vitro	[22]
		A. niger	14.5 mm inhibition zone at 200 µg/mL		
A. annua	artemisinin	*Aspergillus fumigatus*	IC_{50} = 125 µg/mL IC_{90} = 250 µg/mL	in vitro	[23]
A. arborescens	essential oil (chamazulene, camphor)	*Rhizoctonia solani*	47.2% inhibition at 12.5 µL/20 mL medium 100% inhibition at 50 µL/20 mL medium	in vitro	[24]

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
A. argyi leaves	essential oil (caryophyllene oxide, neointermedeol, borneol, α-thujone, β-caryophyllene)	*Aspergillus niger*	MIC = 6.25 µL/mL	in vitro	[25]
A. argyi inflorescence	essential oil (spathulenol, juniper camphor, caryophyllene oxide, terpineol, 1,8-cineole, borneol, camphor, chamazulene)	*Alternaria alternata*	84.7% inhibition at 1000 mg/L	in vitro	[26]
		Botrytis cinerea	93.3% inhibition at 1000 mg/L		
A. austriaca fresh aerial parts	essential oil (camphor)	*Sclerotinia sclerotiorum*	MIC = 2400 µL/L	in vitro	[17]
A. caerulescens ssp. *densiflora*	essential oil (terpinen-4-ol, p-cymene, γ-terpinene, 1,8-cyneole, α-terpineol)	*Alternaria* spp.	20 mm inhibition zone at 1:2 dilution	in vitro	[27]
		Aspergillus spp.	12 mm inhibition zone at 1:1 dilution		
		Fusarium spp.	16 mm inhibition zone at 1:8 dilution		
A. campestris aerial parts	methanol extracts (1:10)	*Aspergillus niger*	32.5–33.1 mm inhibition zone at 20 µg/mL	in vitro	[28]
A. campestris aerial parts	essential oil (α-pinene, β-pinene, β-myrcene, germacrene D)	*Aspergillus flavus*	MIC = 2.5 µL/mL MFC = 2.5 µL/mL	in vitro	[29]
		Aspergillus niger	MIC = 10 µL/mL MFC >20 µL/mL		
		Aspergillus ochraceus	MIC = 2.5 µL/mL MFC = 5 µL/mL		

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
		Aspergillus parasiticus	MIC = 2.5 µL/mL MFC = 5 µL/mL		
		Fusarium culmorum	MIC = 2.5 µL/mL MFC = 5 µL/mL		
		Fusarium graminearum	MIC = 1.25 µL/mL MFC = 1.25 µL/mL		
		Fusarium moniliforme	MIC = 2.5 µL/mL MFC = 2.5 µL/mL		
		Penicillium citrinum	MIC = 5 µL/mL MFC > 20 µL/mL		
		Penicillium expansum	MIC = 2.5 µL/mL MFC = 2.5 µL/mL		
		Penicillium viridicatum	MIC = 10 µL/mL MFC > 20 µL/mL		
A. chamaemelifolia aerial parts	essential oil (carvacrol, thymol, p-cymene α-cadinol)	*Aspergillus oryzae*	MIC = 312.5 µg/mL MFC = 312.5 µg/mL	in vitro	[30]
		Aspergillus niger	MIC = 2500 µg/mL MFC = 2500 µg/mL		
		Byssochlamys spectabilis	MIC = 625 µg/mL MFC = 625 µg/mL		
		Paecilomyces variotii	MIC = 625 µg/mL		

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
			MFC = 625 µg/mL		
		Penicillium chrysogenum	MIC = 625 µg/mL MFC = 625 µg/mL		
		Trichoderma harizanum	MIC = 312.5 µg/mL MFC = 312.5 µg/mL		
A. dracunculus fresh aerial parts	essential oil (sabinene)	*Sclerotinia sclerotiorum*	MIC = 2400 µL/L	in vitro	[17]
A. dracunculus var. *pilosa* fresh aerial parts	essential oil (borneol)		MIC = 2400 µL/L		
		Fusarium moniliforme	MIC = 0.5%		
A. herba-alba aerial parts	essential oil (davanone, camphor, thujone)	*Fusarium oxysporum*	MIC = 0.5%	in vitro direct contact	[31]
		Fusarium solani	MIC = 0.75%		
		Stemphylium solani	MIC = 0.75%		
A. herba-alba leaves	essential oil (β-thujone, α-thujone camphor)	*Penicillium aurantiogriseum*	100% inhibition at 0.89%	in vitro	[32]
		P. viridicatum	100% inhibition at 1.33%		
A. herba-alba fresh leaves	essential oil	*Mucor rouxii*	100% inhibition at 1000 µg/mL	in vitro	[33]
		Penicillium citrinum	100% inhibition at 1000 µg/mL		
	carvone	*Mucor rouxii*	IC_{50} = 7 µg/mL		
		Penicillium citrinum	IC_{50} = 5 µg/mL		

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
A. herba-alba aerial parts	piperitone	*Mucor rouxii*	IC_{50} = 1.5 µg/mL	in vitro	[34]
		Penicillium citrinum	IC_{50} = 2 µg/mL		
	chloroform-methanol extract	*Fusarium solani*	MIC = 62.5 µg/disc		
	11-epiartapshin		MIC = 50 µg/disc		
	santolinylol-3-acetate		MIC = 300 µg/mL		
A. incisa aerial parts	santolinylol	*Aspergillus flavus*	MIC = 300 µg/mL	in vitro	[35]
	trans-ethyl cinnamate		MIC = 500 µg/mL		
	isofraxidin		MIC = 400 µg/mL		
	eupatorin		MIC = 1000 µg/mL		
	scopoletin		inactive		
	esculetin		inactive		
A. judaica aerial parts	essential oil (piperitone, 3-bornanone)	*Aspergillus niger*	MIC = 1.25 µg/disc	in vitro	[36]
		Fusarium solani	MIC = 2.5 µg/disc		
		Fusarium moniliforme	MIC = 2000 µL/L		
A. khorasanica aerial parts	essential oil (davanone, p-cymene, Z-citral, β-ascaridol, thymol)	*Fusarium solani*	MIC = 1500 µL/L	in vitro	[37]
		Rhizoctonia solani	MIC = 1000 µL/L		
		Tiarosporella phaseolina	MIC = 2000 µL/L		

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
A. lavandulaefolia aerial parts	essential oil (eucalyptol, (-)-terpinen-4-ol, α-terpineol)	*Alternaria solani*	EC_{50} = 10.45 mg/mL	in vitro agar diffusion	[20]
			EC_{50} = 6.64 mg/mL	in vitro spore germination	
A. lerchiana fresh aerial parts	essential oil (eucalyptol)	*Sclerotinia sclerotiorum*	MIC = 2400 µL/L	in vitro	[17]
A. maritima aerial parts	essential oil (1,8-cineole, chrysanthenone, germacrene D, borneol)	*Aspergillus flavus*	35.4% inhibition at 10 µL/plate	in vitro	[38]
		A. niger	60.6% inhibition at 10 µL/plate		
		A. ochraceus	56.1% inhibition at 10 µL/plate		
		A. parasiticus	32.45% inhibition at 10 µL/plate		
		A. terreus	58.3% inhibition at 10 µL/plate		
		Fusarium moniliforme	33.9% inhibition at 10 µL/plate		
		Penicillium chrysogenum	28.6% inhibition at 10 µL/plate		
A. nilagirica shoot	essential oil (camphor, β-caryophyllene, α-thujone, sabinene)	*Aspergillus flavus*, *A. niger*, *A. ochraceus*	MIC = 0.29 µL/mL MFC = 0.58 µL/mL	in vitro	[39]
			100% mycotoxin inhibition at 0.16 µL/mL		
		Aspergillus terreus, *Cladosporium cladosporioides*, *Fusarium moniliforme*, *Fusarium*	100% inhibition at 0.29–0.58 µL/mL	in vitro	

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
		oxysporum, Mucor mucedo, Penicillium expansum, P. funiculosum, Rhizopus stolonifer	0% disease incidence at 300 µL/2 L	in situ fumigation test on grapes, 10 days storage	
		Aspergillus flavus toxigenic strain	MIC = 1.4 µL/mL MFC = 4.0 µL/mL	in vitro	
A. nilagirica aerial parts	essential oil (1,5-heptadiene-4-one,3,3,6-trimethyl, artemisia alcohol, α-ionone, benzene, methyl (1-methylethyl))	*Alternaria alternata, Aspergillus flavus, A. minutus, A. niger, A. sydowii, A. terreus, Cheatomium spirale, Curvularia lunata, Mucor* spp., *Mycelia sterilia Penicillium italicum, P. purpurogenum, Rhizopus stolonifer*,	70–100% inhibition at 1.4 µL/mL	in vitro	[40]
			71% protection from fungal contamination at 1.4 µL/mL in air	in situ on *Eleusine coracana* seeds, 12 months storage	
		Macrophomina phaseolina	ED_{50} = 93.23 mg/L		
A. nilagirica aerial parts	essential oil (α-thujone, β-thujone, germacrene D, 4-terpineol, β-caryophyllene, camphene, borneol)	*Rhizoctonia solani*	ED_{50} = 85.75 mg/L	in vitro	[41]
		Sclerotium rolfsii	ED_{50} = 87.63 mg/L		
A. nilagirica leaves	essential oil (α-thujone, borneol, β-thujone, 1,8-cineole)	*Phytophthora capsici*	100% inhibition at 100 ppm	in vitro	[42]
A. pallens leaves	methanol extract 1:10	*Sclerospora graminicola*	Inhibition of zoosporangium formation	in vitro	[43]
A. parviflora twigs	methanol extract 1:1	*Sclerospora graminicola*	Inhibition of zoosporangium		

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
			formation		
A. pontica fresh aerial parts	essential oil (eucalyptol)	*Sclerotinia sclerotiorum*	MIC = 2400 µL/L	in vitro	[17]
A. proceriformis fresh leaves	essential oil (α-thujone)	*Aspergillus carbonarius*	MIC = 10.6 mg/mL	in vitro	[44]
		Aspergillus niger	MIC = 21.2 mg/mL		
		Fusarium graminearum	MIC = 10.6 mg/mL		
		F. verticillioides	MIC = 10.6 mg/mL		
		Septoria glycines	MIC = 2.7 mg/mL		
		Septoria tritici	MIC = 2.7 mg/mL		
A. santonica fresh aerial parts	essential oil (α-thujone)	*Sclerotinia sclerotiorum*	MIC = 2400 µL/L	in vitro	[17]
A. scoparia aerial parts [20]	essential oil (acenaphthene, curcumene, (+) caryophyllene oxide, spathulenol, methyl eugenol, β-caryophyllene)	*Alternaria solani*	EC_{50} = 12.2 mg/mL	in vitro agar diffusion	[20]
			EC_{50} = 3.8 mg/mL	in vitro spore germination	
A. sieberi aerial parts	1*R*, 8*S*-dihydroxy-11*R*,13-dihydrobalchanin [40]	*Fusarium solani*	6 mm inhibition zone at 200 µg/10 µL	in vitro	[45]
	11-epiartapshin [18]		7 mm inhibition zone at 200 µg/10 µL		
	3′-hydroxygenkwanin		8 mm inhibition zone at 200 µg/10 µL		
A. sieberi aerial parts	essential oil (camphor, 1,8-cineole [25])	*Botrytis cinerea*	100% inhibition at 1000 µl/L	in vitro	[46]

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essential oil, the method of extraction—microwave-assisted hydrodistillation and classical hydrodistillation—had no influence on the inhibitory effect against the tested fungi. Both oils contained the same major compounds in comparable ratio [30].

Artemisia Species	Extract * or Compound Tested	Fungi	Inhibitory Dose	Type of Study	Reference
	cineole, camphene, chrysanthenone)				
A. stricta f. *stricta* aerial parts	essential oil (capillene, spathulenol, β-caryophyllene)	*Aspergillus flavus, Aspergillus niger, Sporothrix schenckii* [22]	MIC = 0.625 mg/mL	in vitro	[47]
A. terrae-albae leaves	camphor, 1,8-cineole, camphene, β-thujone [20]	*Aspergillus carbonarius*	MIC > 1.20 mg/mL	in vitro	[48]
		Aspergillus niger	MIC > 1.20 mg/mL		
		Fusarium graminearum	MIC = 0.60–1.20 mg/mL		
		Fusarium verticillioides	MIC = 0.60 mg/mL		
A. turanica aerial parts	essential oil (1,8-cineol, cis-verbenyl acetate, camphor) [32][42]	*Aspergillus niger*	68.6% inhibition at 1 µL/mL [42]	in vitro	[49]
A. vulgaris whole plant [21]	crude methanol extract (1:10)	*Botrytis cinerea*	60% inhibition at 2 mg/mL	in vivo on *Cucumis sativus*	[50]
		Blumeria graminis [48] f. sp. *hordei*	25% inhibition at 2 mg/mL	in vivo on *Hordeum sativum*	
		Magnaporthe grisea [33]	16% inhibition at 2 mg/mL	in vivo on *Oryza sativa*	
		Phytophthora infestans	32% inhibition at 2 mg/mL	in vivo on *Lycopersicon esculentum*	
		Puccinia recondita	52% inhibition at 2 mg/mL [35]	in vivo on *Triticum aestivum*	
		Thanatephorus cucumeris	9.3% inhibition at 2 mg/mL	in vivo on *Oryza sativa*	
A. vulgaris leaves	methanol extract 1:1	*Sclerospora graminicola*	Inhibition of zoosporangium formation [21]	in vitro	[43]

different susceptibility to varied antifungal compounds: for example, *Fusarium solani* was moderately sensitive to the action of isolated substances from *A. sieberi* (two sesquiterpene lactones and one methoxylated flavone), while *Alternaria alternata* and *Aspergillus niger* were resistant [45]. In an analogous manner, Aspergillus niger was

Artemisia Species	Extract * or Compound Tested		Fungi	Inhibitory Dose	Type of Study	Reference
A. vulgaris fresh aerial parts	essential oil (germacrene D)	[28]	*Sclerotinia sclerotiorum*	MIC = 2400 µL/L	in vitro	[17]

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Few studies assessed the antifungal activity in vivo. Ma et al. [21] showed that the petroleum ether extract of *A. annua*, imitating the composition of the essential oil, decreased the incidence of infected *Panax notoginseng* plants when added in the culture mixture. *A. vulgaris* crude methanol extract exhibited weak to moderate antifungal activity against *Magnaporthe grisea, Thanatephorus cucumeris, Botrytis cinerea, Phytophthora infestans, Puccinia recondite,* and *Blumeria graminis* when tested on plants grown in greenhouse conditions [50].

* To highlight the active compounds, the major constituents of the volatile oils were noted in parentheses.

Stored foods can be degraded by fungi such as *Alternaria* spp., *Penicillium* spp., and *Mucor* spp., which reduce their quality and make them unsuitable or even toxic for consumption. The use of chemical products for the control of postharvest pathogens endangers the environment, human health, and can induce resistance to fungicides. Such being the case, some investigations tried to estimate the reduction of postharvest fungal spoilage after treatment with *Artemisia* extracts. Fumigation of table grapes with *A. nilagirica* essential oil (200–300 µL) decreased the weight loss, berry shrinkage, and berry browning, increasing the shelf life for up to 10 days [39]. In addition, *A. nilagirica* volatile oil at a concentration of 1.4 µL/mL in airtight containers provided 71% protection from fungal contamination after 12 months of storage to millet grains [40].

In addition to the direct inhibition of postharvest phytopathogenic fungi, some studies also evaluated the mycotoxins suppression ability of plant extracts. For instance, *Artemisia herba-alba* keto-rich essential oil completely inhibited the toxin production (penicillic acid, terrestric acid, brevianamide A, aurantiamine, xanthomegnin) for *P. aurantiogriseum* at 0.44% and for *P. viridicatum* at 0.22% [32]. Similarly, *Artemisia nilagirica* essential oil inhibited the production of aflatoxin B_1 by *Aspergillus flavus* toxigenic strain at 1 µL/mL. A common seed contaminant, aflatoxin B1 is a powerful human carcinogen and a serious health risk; it also contributes to food deterioration by lipid peroxidation [40]. In another experiment, *A. nilagirica* volatile oil (0.16 µL/mL) completely inhibited the production of aflatoxin B_1 by *Aspergillus flavus* and ochratoxin A by *A. niger* and *A. ochraceus* [39].

The phytocompounds mechanism of action against fungi involves the inhibition of enzymes that control energy or structural compounds production, degeneration of fungal cell wall with loss of cytoplasm, and plasma membrane dysfunction. Due to their lipophilic nature, components of essential oils can penetrate cell walls, increase cellular membranes permeability and disturb the fungal cells metabolism, causing their death [11]. Monoterpenes delay sclerotic differentiation and promote the generation of lipid peroxides, which can lead to cell death, while phenols present in the essential oil bond to the active sites of fungal enzymes through their hydroxyl group [51]

In addition, spore germination and germ tube growth are negatively influenced by terpenes from the essential oil. *A. annua* volatile oil arrested mycelia growth and conidia germination of *Fusarium oxysporum* and *Fusarium solani* [21]. Electron microscope observations proved that *A. argyi* essential oil affected the cell morphology and the structure of cell walls in *Aspergillus niger* [25]. An earlier study showed that *Artemisia herba-alba* essential oil

inhibited mycelium growth, spore germination, and sporulation of *Zygorrhynchus* spp., *Aspergillus niger* and *Penicillium italicum* [52].

The antifungal mode of action of *A. nilagirica* essential oil was investigated by Kumar et al. The fungal cells treated with 1.4 µL/mL volatile oil exhibited important deformity and shrinkage, detachment of plasma membrane from the cell wall, and development of lomasomes. At the same dose, *A. nilagirica* essential oil completely inhibited ergosterol synthesis in the cell membrane of *Aspergillus flavus* and provoked the leakage of Ca^{2+}, K^{+}, and Mg^{2+} ions from the cell [40].

It is worth mentioning that in addition to the secondary antifungal metabolites produced by plants, certain endophytic organisms present in *Artemisia* species are able to inhibit the development of phytopathogenic fungi. Thus, in the root, stem, and leaves of *A. argyi*, researchers identified endophytes (*Bacillus subtilis, B. cereus, Paenibacillus polymyxa*) that produce substances capable of inhibiting the growth of the mycelium of *Fusarium oxysporum, Magnaporthe grisea*, and *Alternaria alternata* [53].

2.2. Antibacterial Activity

Only a small number of studies investigated the effect of *Artemisia* spp. extracts on phytopathogenic bacteria. For instance, different *A. nilagirica* leaves extracts were tested in vitro against four phytopathogenic bacteria, *Erwinia* spp., *Clavibacter michiganense*, *Pseudomonas syringae,* and *Xanthomonas campestris*, which cause diseases in potato, tomato, leafy greens, carrot, onion, and green pepper. The hexane extract was the most efficient in inhibiting all tested bacteria with MIC of 32 µg/mL. The ethanol, methanol, diethyl ether, and chloroform extracts were moderately active against the four bacteria, while the petroleum ether extract was the least effective [54]. Methanol, ethanol, and chloroform extracts from leaves of *Artemisia parviflora* (1:6 *w*/*v*) were almost ineffective against *Xanthomonas vesicatoria* and *Ralstonia solanacearum*, with inhibition zones of 1 and 2 mm [55].

The essential oil of *Artemisia turanica* exhibited inhibitory activity at 2% (*v*/*v*) concentration against tumor galls induced by *Agrobacterium tumefaciens* on potato discs, but it did not demonstrate antibacterial activity in vitro against *A. tumefaciens* at the same dose [49]. In addition, the methanol extracts of roots, leaves, and flowers of *Artemisia fragrans* inhibited tumor growth in different percentages at 10, 100, and 1000 ppm. Leaves and flowers extract had the highest inhibition at all concentration (20, 38, 46%) compared to root extract (15, 24, 34%). No extract had any significant effect on the viability of *A. tumefaciens* when tested by agar diffusion assay [56].

Dadasoglu et al. [57] evaluated the antibacterial activities of essential oils, hexane, chloroform, acetone, and methanol extracts from the aerial parts of *A. santonicum*, *A. spicigera,* and *A. absinthium* against 25 plant pathogenic bacterial strains. *A. spicigera* essential oil was only active (MIC = 500 µL/mL) against *Erwinia amylovora*, *Pseudomonas syringae* pv. *syringae,* and *Xanthomonas axonopodis* pv. *vesicatoria*. The volatile oil of *A. absinthium* exhibited moderate activity (MIC = 250–500 µL/mL) against most of the phytopathogenic bacteria. *A. santonicum* essential oil was the most effective with MIC values 125–250 µL/mL on 22 out of 25 bacteria tested, with the exception of *Pseudomonas aeruginosa*, *P. cichorii,* and *Clavibacter michiganensis* subsp. *michiganensis*. None of the *Artemisia* solvent extracts manifested antibacterial activity on the tested strains. The main constituents

of *A. absinthium* oil were chamazulene, nuciferol butanoate, nuciferol propionate, and caryophyllene oxide, while *A. santonicum* and *A. spicigera* oil shared similar major components: camphor, 1,8-cineole, cubenol, borneol, terpinen-4-ol, and α-terpineol.

In the previously mentioned study, some constituents isolated from the essential oils were evaluated individually for their antibacterial activity. Caryophyllene oxide, camphor, borneol, and 1,8-cineole did not show activity against the phytopathogenic bacteria. Terpinen-4-ol inhibited the growth of all tested bacteria with MIC values ranging from 60 to 110 µL/mL and linalool blocked the development of 22 bacterial strains with MIC values in the 50–110 µL/mL domain. α-Terpineol was active (MIC = 60–70 µL/mL) only on *Pseudomonas cichorii*, *P. huttiensis*, *P. syringae* pv. *syringae,* and *Xanthomonas axonopodis* pv. *vesicatoria* [57].

The essential oil extracted from fresh leaves of *Artemisia proceriformis* manifested weak antimicrobial activity against four bacteria: *Erwinia carotovora* (MIC = 21.2 mg/mL), *Pseudomonas corrugate* (MIC = 21.2 mg/mL), *Pseudomonas syringae* (MIC = 5.31 mg/mL), and *Xanthomonas vesicatoria* (MIC > 42.5 mg/mL). The major component was α-thujone, in proportion of 66.9% [44].

Terpenes and phenolic compounds found in the essential oils are responsible for the intense antimicrobial activity. Terpenes have the ability to increase membrane permeability by infiltrating the phospholipidic bilayer; the damage to the bacterial membrane causes the loss of cytoplasmic components, which leads to cell death. Plant extracts are studied not only as inhibitors of bacterial growth, but also for the prevention of biofilm formation. Such is the case of *A. herba-alba*, *A. absinthium,* and *A. campestris* essential oils that can reduce biofilm formation by up to 70% [58].

2.3. Insecticidal Activity

Insects are the more diverse group of animals on Earth, and only 0.5% are considered pests. Nonetheless, herbivorous insects destroy every year one-fifth of the world's crop production. Synthetic chemicals used to control insect pests are toxic to humans, animals, and the environment through accumulation. In addition, the development of insecticide resistance and the migration of harmful insects require the search for an alternative for plant protection. Considering these facts, botanical insecticides represent a viable substitute with low toxicity toward humans and the environment [59].

Plant-derived substances or plant extracts usually have a lower acute toxicity toward insects compared to synthetic insecticides. Nevertheless, their subacute toxicity was frequently noted and is important because it can limit insect spreading (diminished fertility, fecundity, vitality, or shorter lifespan) and decrease crop loss due to repellent, suppressant, or deterrent activity. These effects are generally called "antifeedant" and are manifested in insects by lower weight and body size, decreased fertility, and altered behavior [60].

Artemisia compounds can influence insects by direct contact or fumigation, can repel insects or keep them from feeding, or can hinder their reproduction. Volatile compounds can induce toxicity to insects via inhalation or direct contact by forming an impermeable film on the cuticle leading to suffocation. Some volatile components can penetrate through the cuticle, affecting cellular membrane function and oxidative phosphorylation [61].

Phytochemicals such as cinnamyl alcohol, eugenol, and trans-anethole can activate octopamine receptors, interfering with the normal activity of octopamine, a neurotransmitter, neuromodulator, and neurohormone in an invertebrate system [62]. Furthermore, volatile compounds can interfere with the γ-aminobutyric acid (GABA) receptor in insects [14]. Other studies reported the inhibition of acetylcholinesterase by 1,8-cineole, (-)-citronellal, limonene, α-pinene, pulegone, and 4-terpineol [63] or inhibition of adenosinetriphosphatase by essential oils [64]. In addition, plant substances may cause the suppression of cytochrome P450 in insects (the enzymes responsible for phase I metabolism of xenobiotics) and may alter various biochemical processes, which shift the balance of the endocrine system [14].

The activity of *Artemisia* compounds and extracts depends on the solvent used, the susceptibility of pest species to the active substance, the development stage of the insect, whether it is male or female, and the method of application. Table 2 lists the more recent studies on insecticidal activity of *Artemisia* genus. Essential oils and volatile compounds can be applied via fumigation, which is a procedure used frequently in the pest management of stored products. This method has obvious advantages such as the possibility to spread the substance evenly, even in unreachable places, and the ability to maintain an effective level of insecticides within a closed space [60]. Some of the shortcomings of natural insecticides are poor water solubility and rapid degradation in the environment, leading to low persistence and poor efficiency. To solve these problems, plant insecticides may be formulated as micro- and nanocapsules, nanoparticles, or nanoemulsions. These nanoformulations can increase the solubility, persistence, and stability of bioinsecticides, enhancing their activity and, at the same time, limiting their negative impact on the environment [65].

Table 2. Insecticidal activity of *Artemisia* compounds and extracts.

Artemisia spp.	Extract or Compound Tested	Target Species	Reference
A. absinthium	essential oil	*Leptinotarsa decemlineata* *Myzus persicae* *Rhopalosiphum padi* *Spodoptera littoralis*	[18]
	essential oil	*Trialeurodes vaporariorum* *Tuta absoluta*	[66]
	essential oil	*Tetranychus cinnabarinus*	[67]
	essential oil	*Diaphania hyalinata*	[68]
	methanol extract	*Sitophilus oryzae*	[69]
	essential oil	*Orysaephilus surinamensis* *Tribolium castaneum*	[70]
	powdered plant	*Oryzaephilus surinamensis*	[71]
	water extract	*Hyphantria cunea*	[72]

Artemisia spp.	Extract or Compound Tested	Target Species	Reference
	ethanol extract		
	supercritical extracts	*Spodoptera littoralis*	[73]
	essential oil	*Myzus persicae*	[74]
	essential oil carvacrol (−)-α-bisabolol chamazulene	*Diaphorina citri*	[75]
A. annua	methanol extract essential oil	*Helicoverpa armigera*	[76]
	methanol extract artemisinic acid artemisinin scopoletin arteannuin-B deoxy-artemisinin artemetin casticin chrysosplenetin	*Helicoverpa armigera*	[77]
	essential oil	*Glyphodes pyloalis*	[78]
	methanol extract	*Pieris rapae*	[79]
	methanol extract	*Hyphantria cunea*	[80]
	methanol extract	*Glyphodes pyloalis*	[81]
	essential oil	*Diaphania hyalinata*	[68]
A. arborescens	essential oil	*Rhysopertha dominica*	[24]
A. argyi	ethanol extract	*Brevicoryne brassicae*	[82]
A. frigida	essential oil	*Diaphania hyalinata*	[68]
	water extract ethanol extract	*Hyphantria cunea*	[72]
	essential oil	*Plodia interpunctella*	[83]
	essential oil	*Liposcelis bostrychophila* *Sitophilus zeamais*	[84]
	essential oil terpinen-4-ol	*Lasioderma serricorne* *Liposcelis bostrychophila*	[85]

Artemisia spp.	Extract or Compound Tested	Target Species	Reference
	verbenone camphene α-terpineol α-terpinyl acetate	*Tribolium castaneum*	
A. herba-alba	essential oil	*Orysaephilus surinamensis* *Tribolium castaneum*	[70]
A. judaica	essential oil	*Sitophilus orizae*	[64]
A. lavandulaefolia	essential oil 1,8-cineole chamazulene β-caryophyllene	*Lasioderma serricorne*	[86]
A. monosperma	essential oil	*Sitophilus orizae*	[64]
	essential oil	*Aphis nerii*	[87]
A. nilagirica	cow urine extract	*Scirpophaga incertulas*	[88]
A. spicigera	essential oil	*Dendroctonus micans*	[89]
A. vulgaris	essential oil	*Callosobruchus maculatus* *Rhyzopertha dominica* *Tribolium castaneum*	[90]
	essential oil	*Diaphania hyalinata*	[68]
	water extract ethanol extract	*Hyphantria cunea*	[72]

2.4. Nematicidal Activity

Plant parasitic nematodes cause severe yield losses in different crops, especially in tropics and subtropics. Frequent nematodes that affect plants include *Meloidogyne* (root-knot nematodes), *Pratylenchus* (lesion nematodes), *Xiphinema* (dagger nematodes), *Aphelenchoides* (foliar nematodes), *Globodera* (potato cyst nematodes), and *Heterodera* (soybean cyst nematodes). *Meloidogyne* species induce histological damages to roots, with the appearance of visible galls. Some phytoparasitic nematodes act as vectors for plant viruses, such as *Xiphinema* species [91].

Various *Artemisia* species were evaluated for nematicidal activity, some with promising results. For instance, *A. judaica* essential oil (1 µL/L) caused 85% mortality on *Meloidogyne javanica* second-stage juveniles and inhibited the hatching of eggs. The main component of the essential oil was artemisia ketone. In the same study, *A. arborescens* and *A. dracunculus* essential oils were poorly active on the root-knot nematode [92]. In vitro toxicity of *Artemisia annua* essential oil was evaluated against second-stage juveniles of *Meloidogyne incognita* and pre-

adults of *Rotylenchulus reniformis* (reniform nematode). Concentrations of 500 and 250 ppm induced 100% mortality in both nematode species [93]. Moreover, there are reports of nematicidal activity exhibited by the alcoholic and aqueous extracts of *Artemisia annua* against *Meloidogyne incognita* and *Pratylenchus loosi* (tea root lesion nematode) [91].

Artemisia herba-alba essential oil produced 94.4% mortality on *Meloidogyne incognita* second-stage juveniles at 15 µg/mL and 100% mortality on *Xiphinema index* females at 2 µg/mL, after 24 h exposure. However, mixed-age infective specimens of *Pratylenchus vulnus* were more resistant to the activity of *A. herba-alba* essential oil with mortality values ranging from 56.8% to 67% after 24 to 96 h of exposure. The major components of the essential oil were cis- and trans-thujone, camphor, 1,8-cineole, trans-chrysantenyl acetate, and camphene. In an additional test, the three nematode species were exposed to various compounds of the essential oils of four plants, including *A. herba-alba*. Borneol and α-pinene manifested poor to moderate activity, while limonene lack activity on the three nematode species. Camphor exhibited a moderate nematicidal effect, whilst thymol and thujone (mixture of cis-thujone, 70% and trans-thuione) displayed strong activity against *M. incognita*, and less so on *P. vulnus* and *X. index*. The fact that the activity of the components of the volatile oil is weaker than that of the whole oil suggests a possible synergistic action of the mixture. In addition, soil treatments with 100 or 200 µg/kg *A. herba-alba* essential oil, by fumigation or application of water solution, significantly inhibited nematode density on tomato roots and in soil and also increased the plant biomass. Fumigation was proven to be more effective than drenching treatment [94].

A. absinthium essential oil (β-thujone 51% and linalyl acetate 24%) had over 99% mortality rate at 0.25 and 0.5% concentrations (*v/v*) against *Meloidogyne javanica* juveniles in an in vitro test. Furthermore, in vivo experiments were conducted in order to assess the ability of the essential oil to inhibit root-knot nematode development after being absorbed by the tomato plants. It was observed that spraying the oil on tomato leaves actually increased the number of galls and eggs in treated plants, and applying the essential oil into the soil at 0.25% and 0.5% concentrations did not lower the number of galls or nematode eggs in tomato plants. The authors believe that the nematicidal compounds could have been volatilized or degraded by microorganisms in the soil or by the plant, or possibly, the root exudates were modified by the absorbed essential oil, making the tomato plants more appealing to the nematodes [95]. In another study, commercially available *A. absinthium* volatile oil had only a slight effect on *Meloidogyne javanica* in vitro (the median lethal dose LC_{50} of 937 µg/mL at 48 h and 734 µg/mL at 72 h). The major components of the oil were borneol acetate, β-terpineol, 1,8-cineol, linalool, sabinene, and o-cymene [96].

The nematicidal activity of *Artemisia absinthium* hydrolate, a by-product of essential oil extraction, was evaluated on the root-knot nematode, *Meloidogyne javanica*. The hydrolate caused high mortality of second-stage juvenile and suppression of egg hatching, proving the ability of the *A. absinthium* hydrolate to penetrate the gelatinous matrix of eggs. In vivo tests showed a strong inhibition of juveniles' penetration in the tomato roots. Soil treatment with *A. absinthium* hydrolate (60% and 20% concentrations) significantly reduced the reproductive capacity of root-knot nematode and the infection frequency. The main component of the hydrolate, responsible for the nematicidal activity, was identified as (5Z)-2,6-dimethylocta-5,7-dien-2,3-diol [97].

Kalaiselvi et al. [98] showed that essential oils of *A. nilagirica* plants collected from high and low altitude have different composition and different nematicidal activity against *Meloidogyne incognita* ($LC_{50/48h}$ of 5.75 and 10.23 µg/mL, respectively). α-thujone, α-myrcene, and linalyl isovalerate were the main components of high-altitude *A. nilagirica* volatile oil, while the low-altitude plants produced an oil composed mostly of camphor, caryophyllene oxide, eucalyptol, humulene epoxide II, α-humulene, and β-caryophyllene. Experiments carried out in vivo by soil irrigation with the essential oil revealed that both volatile oils significantly reduced the infection of tomato plant (number of nematode juveniles and eggs) and enhanced plant growth (fresh weight of aerial parts and roots) at 20 µg/mL. Again, the effect was greater for the oil originated from high-altitude *A. nilagirica*. Moreover, the ethanol extract of flowering parts of *A. nilagirica* (1 mg/mL) exhibited nematicidal activity against *Meloidogyne incognita*, as reported by an earlier study [99].

Various hypotheses have been advanced as explanations for the nematicidal effects of essential oils: disruption of cell membrane permeability and obstruction of its functions, irreversible modifications of proteins structures from the nematode surface induced by aldehydes, inhibition of acetylcholinesterase with build-up of neurotransmitter in the central nervous system of the nematode followed by convulsion, paralysis, and death [11]. Research on *A. nilagirica* essential oil ascribe the nematicidal action to an increased generation of intracellular reactive oxygen species, activation of signaling pathway of apoptosis, and DNA damage prompting cell death [98].

In addition to the essential oils and their volatile compounds, few other substances from *Artemisia* genus have been tested for their activity against plant nematodes. Thirteen chemical compounds (apigenin, bonanzin, nepetin, dihydroluteolin, scopoletin, isoscopoletin, benzoic acid, β-sitosterol, γ-sitosterol, betulinic acid, friedelin, linoleic acid, and a long chain ketone) isolated from *Artemisia elegantissima* and *Artemisia incisa* were tested in vitro and in vivo for nematicidal activity against *M. incognita*. All phytochemicals significantly inhibited egg hatching and induced high mortality of second-stage juveniles at the tested concentrations (0.1, 0.2, and 0.3 mg/mL). Isoscopoletin was even more effective than the positive control carbofuran. In addition, application of the compounds as a root drench (0.1 mg/mL) on potted tomato plants caused a marked reduction of galls, galling index, and egg masses on plant roots, numbers of juveniles in the rhizosphere soil, and also improved tomato plant growth parameters (shoot and root length and weight). Isoscopoletin and apigenin were the most active compounds [100].

2.5. Herbicidal Activity

One of the most influential groups of plant secondary metabolites is the allelochemicals. They are released into the environment in order to affect the germination, growth, behavior, survival, and reproduction of competing plants, which is a process better known as allelopathy. They are produced mainly in the plant's roots, seeds, flowers, and leaves, and their synthesis depends on the changes of the climate conditions as well as exposure to biotic or abiotic stress. Allelochemicals activity can be harmful or beneficial for the growth and survival of target species [101]. The destructive effect of allelochemicals is crucial for defending plants against herbivores and providing an advantage in the competition for resources [102]. In agroecosystems, allelopathy can influence weed management,

and plant allelochemicals could be employed as bioherbicides in order to reduce the negative impact of chemical herbicides on the environment [103].

The allelopathic properties of *Artemisia* species are well known [104][105][106][107][108][109][110], so it was expected that numerous studies would investigate their herbicide potential on various weeds. Most researchers focused on the volatile oils, and only a few dealt with aqueous or alcoholic extracts (Table 3). The phytotoxic effect of essential oils is owed to multiple mechanisms of action: inhibition of cell division, decrease of mitochondrial respiration, reduction of photosynthetic pigments and photosynthesis, generation of radical oxygen species in excess and oxidative impairment, destruction of waxy cuticular layer, inhibition of enzymes activity, water uptake, and alteration of gibberellic acid content [102][111][112]. Most of these actions are correlated with the presence of oxygenated monoterpenes. For example,1,8-cineole and camphor inhibit DNA synthesis, cell proliferation, and elongation [113].

Table 3. Phytotoxic activity of *Artemisia* compounds and extracts.

***Artemisia* Species**	**Extract * or Compound Tested**	**Weed/Target Plant**	**Observed Effect**	**Reference**
A. absinthium aerial parts	essential oil (cis-epoxyocimene, (−)-cis-chrysanthenol, chrysanthenyl acetate, linalool and β-caryophyllene)	*Lolium perene*	Suppression of root and leaf growth No effect on seed germination	[18]
		Lactuca sativa	Suppression of root and leaf growth No effect on seed germination	
A. absinthium fresh aerial parts	essential oil (β-thujone, chamazulene)	*Sinapis arvensis*	Complete inhibition of seed germination and seedling growth at 2 µL/mL	[114]
A. absinthium leaves	aqueous extract 1:10 *w/v*	*Parthenium hysterophorus*	Inhibition of seed germination, shoot and root growth, reduction of chlorophyll and carotenoid content, at 25, 50, 75, and 100% Enhanced malondialdehyde levels, phenolic content and increased activity of antioxidative enzymes, at 25, 50, 75, and 100%	[105]
A. absinthium shoot and root	aqueous extract	*Chenopodium album*	Decreases growth criteria (root and shoot length and fresh weight, number of leaves) at 1–	[115]

Artemisia Species	Extract * or Compound Tested	Weed/Target Plant	Observed Effect	Reference
			100 mg/mL No effect on seed germination Increased peroxidase and superoxide dismutase activity in root	
A. afra leaves	aqueous extract	*Triticum aestivum*	No effect on seed germination	[116]
		Brassica napus	Complete inhibition of seed germination	
		Medicago sativa	Increased germination rate	
		resistant and non-resistant *Lolium* spp.	Significant inhibition of seed germination	
A. annua flower heads	essential oil (1,8-cineole, trans-sabinyl acetate, artemisia ketone, camphor α-pinene)	*Amaranthus retroflexus*	In vitro, complete inhibition of seed germination, at 10 and 100 µg/L In vivo, plant death, at the cotyledon stage (100 mg/L) and true leaf stage (1000 mg/L)	[117]
		Setaria viridis	In vitro, complete inhibition of seed germination, at 100 µg/L In vivo, plant death, at the cotyledon stage (100 mg/L) and true leaf stage (1000 mg/L)	
A. annua aerial parts	artemisinin arteannuin B artemisinic acid	*Secale cereale, Hordeum vulgare, Artemisia annua, Portulaca oleracea, Amaranthus blitun, Lactuca sativa, Raphanus sativus*	Inhibition of seed germination Inhibition of shoot and root growth	[118]
A. annua	artemisinin	*Lactuca sativa*	Inhibition of root and shoot elongation, reduced cell division and cell viability in root tips, at 10 µM Reduced chlorophyll a	[119]

Artemisia Species	Extract * or Compound Tested	Weed/Target Plant	Observed Effect	Reference
			and b levels Increased malondialdehyde and proline levels, at 1 µM	
A. annua	artemisinin	*Arabidopsis thaliana*	Reduction of fresh biomass, chlorophyll a, b, and leaf mineral contents at 40–160 µM Reduction of photosynthetic efficiency, yield, and electron transport rate, calcium and nitrogen levels at 80 and 160 µM Elevated lipid peroxidation (malondialdehyde contents) at 80 and 160 µM	[120]
A. arborescens shoot	sesamin ashantin	*Agrostis stolonifera*, *Lactuca sativa*	Growth inhibition at 1 mg/mL	[107]
	sesamin	*Lemna paucicostata*	Growth inhibition IC_{50} = 401 µM	
	ashantin	*Lemna paucicostata*	Growth inhibition IC_{50} = 224 µM	
A. arborescens leaf litter	crude methanol extract	*Lactuca sativa, Raphanus sativus, Amaranthus retroflexus, Cynodon dactylon*	Inhibition of seed germination ED_{50} = 1.61–3.05 mg/mL Inhibition of root growth ED_{50} = 1.22–3.14 mg/mL	[121]
	hexane, chloroform, and ethyl acetate fractions		Inhibition of seed germination ED_{50} = 1.19–6.25 mg/mL Inhibition of root growth ED_{50} = 0.92–3.98 mg/mL	
A. arborescens aerial part	crude methanol and aqueous extracts	*Lactuca sativa*	Inhibition of seed germination and root	[122]

Artemisia Species	Extract * or Compound Tested	Weed/Target Plant	Observed Effect	Reference
			growth ED_{50} = 0.5–2.8 mg/mL	
	ethyl acetate, n-hexane, chloroform, n-butanol fractions		Inhibition of seed germination and root growth ED_{50} = 0.4–5.4 mg/mL	
A. argyi leaves	water extract (caffeic acid, schaftoside, 4-caffeoylquinic acid, 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid and 3-caffeoylquinic acid)	*Brassica pekinensis, Lactuca sativa, Oryza sativa*	Inhibition of germination, root and stem growth, and biomass (at 50, 100, and 150 ng/mL)	[108]
		Brassica pekinensis, Lactuca sativa, Oryza sativa, Portulaca oleracea, Oxalis corniculata, Setaria viridis	Inhibition of germination and growth in pot experiment (*A. argyi* powder mixed into sand soil at the ratio 100:0, 100:2, 100:4, and 100:8)	
A. campestris leaves	essential oil (β-pinene, 1, 8-cineole, p-cymene, myrcene)	*Daucus carota, Cicer arietinum, Phaseolus vulgaris, Triticum sativum*	Reduces seed germination at 1000–2000 ppm Enhances seed germination at 100 ppm Delays the germination of *D. carota* seeds	[123]
A. dracunculus aerial parts	essential oil	*Medicago minima, Rumex crispus, Taraxacum officinale*	No effect on seed germination at 0.3–1.2 mg/L	[124]
A. dracunculus	leachate	*Lactuca sativa*	Radicle growth inhibition	[125]
A. fragrans aerial parts	essential oil (α-thujone, camphor, 1,8-cineole, β-thujone)	*Convolvulus arvensis*	Important reduction in the shoot, root, and plant length, shoot and root fresh weight, shoot and root dry weight Inhibited seed germination Significant decrease of photosynthetic pigments and antioxidant enzymes Increased production of H_2O_2 and malondialdehyde content, and membrane leakage	[126]

Artemisia Species	Extract * or Compound Tested	Weed/Target Plant	Observed Effect	Reference
A. fragrans roots, leaves, and flowers	methanol extracts	*Raphanus raphanistrum*	Inhibition of root growth at 1000 ppm Inhibition of seed germination at 7500 ppm	[56]
A. frigida	volatile organic compounds (1,8-cineole, camphene, (E)-3-hexen-1-ol acetate, α-terpineol, β-terpineol)	*Melitotus suaveolens, Sorghum sudanense, Elymus dahuricus, Agropyron cristatum*	Significantly decreases the seed germination and seedling growth	[127]
A. judaica aerial parts	essential oil (piperitone, 3-bornanone)	*Lactuca sativa*	Reduced seed germination, shoot and root growth at 250–1000 µL/L	[36]
A. lavandulaefolia leaves	aqueous extract	*Lactuca sativa, Artemisia princeps, Achyranthes japonica, Oenothera odorata, Plantago asiatica, Aster yomena, Elsholzia ciliata, Raphanus sativus*	Inhibition of root growth Inhibition of seed germination	[128]
	essential oil (1,8-cineole, α-terpineol, α-terpinene, camphor, azulene, 2-buten-1-ol)			
A. monosperma aerial parts	aqueous extract	*Phaseolus vulgaris*	Stimulation of seed germination at 1% and 2% concentration Inhibition of seed germination at 3% and 4% concentration Inhibition of amylase and protease activity	[129]
A. monosperma aerial parts	aqueous extract	*Medicago polymorpha*	Reduction of germination percentage, plumule and radicle growth, and seedling dry weight	[130]
	crude plant powder mixed with clay loam soil		Inhibitory effects on leaf area index, total photosynthetic pigments, total available carbohydrates and total protein, in pot culture bioassay	
A. scoparia fresh leaves	essential oil (β-myrcene, (+)-	*Avena fatua, Cyperus rotundus, Phalaris*	Important reduction in germination, seedling	[131]

Artemisia Species	Extract * or Compound Tested	Weed/Target Plant	Observed Effect	Reference
	limonene, (Z)-β-ocimene, γ-terpinene)	*minor*	growth, and dry matter at 0.07–0.7 mg/mL	
A. scoparia fresh leaves	essential oil (p-cymene, β-myrcene, (+)-limonene)	*Achyranthes aspera, Cassia occidentalis, Parthenium hysterophorus, Echinochloa crus-galli, Ageratum conyzoides*	Inhibition of seed germination, root and shoot growth at 10, 25, and 50 µg oil/g sand Chlorosis, necrosis and complete wilting of plants 1 to 7-days after spraying with oil (2%, 4%, and 6%, *v*/*v*) Significant decline in chlorophyll content and cellular respiration, electrolyte leakage	[132]
A. sieversiana fresh aerial parts	essential oil (α-thujone, eucalyptol)	*Amaranthus retroflexus, Medicago sativa, Poa annua, Pennisetum alopecuroides*	Inhibition of root and shoot growth IC_{50} = 1.89–4.69 mg/mL	[133]
	α-thujone		IC_{50} = 1.55–6.21 mg/mL	
	eucalyptol		IC_{50} = 1.42–17.81 mg/mL	
	α-thujone and eucalyptol mixture		IC_{50} = 0.23–1.05 mg/mL	
A. terrae-albae aerial parts	essential oil (α-thujone, β- thujone, eucalyptol, camphor)	*Amaranthus retroflexus*	Reduces root and shoot growth at 1.5 µg/mL Completely inhibits seed germination at 3 µg/mL	[134]
		Poa annua	Reduces root and shoot growth at 1.5 µg/mL Completely inhibits seed germination at 5 µg/mL	
A. verlotiorum flower heads	essential oil (chrysanthenone, 1,8-cineole, β-pinene, camphor 2,6-dimethyl phenol, β-caryophyllene)	*Amaranthus retroflexus*	In vitro, complete inhibition of seed germination, at 10 and 100 µg/L In vivo, plant death, at the cotyledon stage (100 mg/L) and true leaf stage (1000 mg/L)	[117]
		Setaria viridis	In vitro, inhibition of seed germination, at 10 and	

Artemisia Species	Extract * or Compound Tested	Weed/Target Plant	Observed Effect	Reference
			100 µg/L In vivo, plant death, at the cotyledon stage (1000 mg/L) and true leaf stage (1000 mg/L)	
A. vulgaris aerial parts	aqueous extract	*Amaranthus retroflexus*	Inhibition of seed germination, radicle, and hypocotyl length at 7.5% to 10% *w/v*, in Petri dish bioassays Inhibition of seedling emergence and plant growth, in pot culture bioassays	[104]
		Zea mays [133]	Stimulation of radicle and mesocotyl growth at 7.5% to 10% *w/v*, in Petri dish bioassays Stimulation of plant biomass, in pot culture bioassays	
A. vulgaris leaves and flowers	essential oil	*Agrostemma githago, Amaranthus* [86] *retroflexus, Cardaria draba, Chenopodium album, Echinochloa crus-galli, Reseda lutea, Rumex crispus, Trifolium pratense*	Inhibition of root and shoot growth and reduction of germination rate (at 2, 5, 10 and 20 µL/plate)	[135]
A. vulgaris root	aqueous extracts	*Triticum aestivum* [134] (winter wheat)	Inhibition of shoot and root growth by all concentrations (1:6250 to 1:10)	[128]
		Brassica napus spp. *oleifera* [132] var. *biennis* (winter oilseed rape)	Significant inhibition of germination at the 1:10 concentration	[136]
A. vulgaris aerial parts			Significant inhibition of root growth at 1:10 concentration Stimulation of shoot growth	

at 1–4% pigments ascorbate xide and e electron n reactive ge [126].

e 64.46% *oa annua,* the same possible

by hydro- ytotoxicity pacts the

th of *Poa* nixture of mponents *dulaefolia* (Table 3),

ve stress erferes in

ract (20% 0%, while

manifesting an inhibitory effect of 70% against *Lolium multiflorum* [137]. Another field trial demonstrated that *A. argyi* water extract markedly suppressed the growth of weeds in *Chrysanthemum morifolium* field with no adverse effect on the growth of *C. morifolium*. The investigations showed that *A. argyi* inhibited weed growth and germination through inhibition of chlorophyll synthesis and photosynthesis [108]. Conversely, field treatment of *Triticum turgidum*

* To highlight the active compounds, the major constituents of the volatile oils were noted in parentheses.

L. subsp. *durum* Desf. with *A. absinthium* aqueous extract exerted a stimulating effect on weed presence and reduced wheat growth and yield [106].

The sensitivity of different weed species to a certain herbicide varies greatly. Among eight weeds tested in a study, *Amaranthus retroflexus, Echinochloa cruss-galli,* and *Reseda lutea* were more susceptible to the action of *A. vulgaris* essential oil, compared to *Rumex crispus, Agrostemma githago, Trifolium pretense, Chenopodium album,* and *Cardaria draba,* which were more resistant [135]. Similarly, *Parthenium hysterophorus* and *Ageratum conyzoides* were more vulnerable to the inhibitory effect of *Artemisia scoparia* volatile oil, in comparison with *Cassia occidentalis*, under laboratory conditions. In another test, *Echinochloa crus-galli* and *Parthenium hysterophorus* were more affected by post-emergence application of the oil [132].

The phytotoxicity of isolated compounds from *Artemisia annua* was evaluated against two monocots and five dicots (Table 3). The suppression of germination and seedling growth varies in the order: artemisinin>arteannuin B>artemisinic acid. *Raphanus sativus* was the most resistant to the action of tested compounds, followed by *Secale cereale*. The weaker activity of arteannuin B and artemisinic acid—molecules without an endoperoxide bridge—implies that the moiety is important for the phytotoxic effect [118]. Artemisinin reduces many physiological and biochemical processes in the target plant and affects mitosis by inhibiting microtubules formation [120][138].

The incorporation of artemisinin into soil inhibited the growth of above-ground lettuce plants, with EC_{50} = 2.5 mg/Kg sandy soil, but the germination was not arrested up to 100 mg/Kg soil [139]. Furthermore, adding *A. annua* leaves containing 0.81–0.22% artemisinin in soil led to the inhibition of *Zea mays* growth [140]. Artemisinin is phytotoxic in concentrations comparable to those of commercial herbicides and has a good activity in soil [110].

In vivo tests proved that artemisinin is a potent suppressor of photosynthetic activity through the formation of a highly reactive artemisinin-metabolite that is able to inhibit the photosynthetic electron flow [141]. Other investigations showed that artemisinin enhances the generation of radical oxygen species and lipid peroxidation, which leads to cell death and arrest of mitotic phases in *Lactuca sativa* seedlings [119]. When added to the culture medium of *Arabidopsis thaliana* seedlings, artemisinin (1, 2, 5, 20, 100 µM) reduced the root gravitropic responses, elongation of primary and lateral roots, root hairs density, and length. Furthermore, artemisinin diminished starch grain and auxin concentrations and affected auxin redistribution in root tips [142].

2.6. Activity on Non-Target Organisms

Since biopesticides and bioherbicides are of natural origin, they are considered to be less harmful to the environment and the health of applicators and consumers. Usually, plant-based formulations are mixtures of compounds, and they do not consist of a single substance, which should prevent resistance in target organisms. In addition, some phytochemicals are rapidly degraded in nature, so there is no risk of their accumulation in the environment, as is the case with chemical pesticides. Consequently, plant-based pesticides and herbicides are regarded as generally safe. Still, these products can affect the non-target organism directly or indirectly by influencing biodiversity and species interactions, so it is imperative to assess their safety [13][143].

Little information is available regarding the ecotoxicity of *Artemisia* compounds and extracts. Pino-Otin et al. [13] evaluated the toxicity of hydrolate and organic extracts from *A. absinthium* on three aquatic ecotoxicity indicator organisms: an invertebrate (*Daphnia magna*), a marine bacterium (*Vibrio fisheri*), and a unicellular freshwater alga (*Chlamydomonas reinhardtii*). The wormwood hydrolate, a by-product of essential oil extraction, is a promising biopesticide with nematicidal effect due to (5*Z*)-2,6-dimethylocta-5,7-dien-2,3-diol [97]. *A. absinthium* hydrolate caused acute toxicity on non-target organisms: *D. magna* (LC_{50} = 0.236%) > *V. fisheri* (LC_{50} = 1.85%) > *C. reinhardtii* (LC_{50} = 16.49%). Moreover, the wormwood ethanol extract was highly toxic to *D. magna* (LC_{50} = 0.093 mg/L). However, the effect of wormwood hydrolate on a river microbial community, composed mainly of Proteobacteria, was negligible, causing only small changes in metabolic diversity and a slight inhibition of bacterial growth. It is possible that natural freshwater microbial populations are more resistant to 2,6-dimethylocta-5,7-diene-2,3-diol action because of the modified bioavailability of compounds in the river water and particular sensitivity of the various microbial species [13].

The same *A. absinthium* hydrolate was tested on non-target soil organisms: natural microbial communities, the earthworm *Eisenia fetida*, and the plant *Allium cepa*. The hydrolate was toxic in low concentrations: it caused substantial inhibition of onion root growth (LC_{50} = 3.87% *v/v*), high mortality of the earthworm *E. fetida* (LC_{50} = 0.07 mL/g), and decreased bacterial metabolism (LC_{50} = 25.72% *v/v* after 1 day of exposure). All these effects were exhibited at inferior concentrations than those needed to contain the target organism. Probably, 2,6-dimethylocta-5,7-diene-2,3-diol is able to penetrate biological membranes and thus affect the survival and metabolic processes of soil organism from different trophic levels [13].

The methanol extracts of *Artemisia fragrans* manifested significant toxicity in the brine shrimp (Artemia salina) lethality assay, with ED_{50} = 19.7 ppm for the root extract and ED_{50} = 11.99 ppm for flowers and leaves extract [56]. In another study, the aqueous extracts from *Artemisia ordosica* leaves were tested on two algae from the biological soil crusts, *Chlorella vulgaris* and *Nostoc* spp. The less concentrated extract (1 g/L) stimulated *C. vulgaris* growth but did not significantly affect *Nostoc* spp., indicating that *C. vulgaris* might utilize the sugars and other carbon sources in the extract to promote self-growth. The highly concentrated extract (5 and 10 g/L) inhibited the growth of both algae [109].

The safety profile of the *Artemisia nilagirica* essential oil was determined in terms of mammalian toxicity on male mice (*Mus musculus*) and millet (*Eleusine coracana*) seeds viability. The essential oil showed low toxicity on mice (LD_{50} = 7528.10 µL/kg) and no effect on millet seed germination. Thus, the oil is suitable as a food preservative for both consumption and sowing purposes [40]. More so, *Artemisia nilagirica* essential oil did not cause any significant changes in the physicochemical and sensory properties of table grapes when applied by fumigation on the fruits [39].

Artemisia absinthium essential oil, a potential biopesticide, was evaluated for toxicity against non-target organisms: the honey bee (*Apis mellifera*) and tomato plant (*Solanum lycopersicum*). Honeybee toxicity (EC_{50} = 0.26 mg/cm^2) is reached at lower concentrations of *A. absinthium* oil than the ones necessary for controlling the leaf miner *Tuta absoluta* (EC_{50} = 0.5 mg/cm^2), but not at rates needed to control the whitefly *Trialeurodes vaporariorum* (EC_{50} =

0.08 mg/cm^2). A similar phenomenon was noted for the phytotoxic effect on tomato; seed germination and root growth were inhibited at oil concentrations needed to control the leaf miner, but not the whitefly [66].

Investigations to date have shown that biopesticides derived from *Artemisia* are most likely to have some toxicity toward non-target organisms, and further studies are needed to assess the risk in natural communities in order to ensure the safe use of biopesticides in agricultural practices.

Choosing the right formulation can reduce toxicity as well as increase the stability and effectiveness of *Artemisia* biopesticides. For instance, terpenoids are lipophilic, volatile, and thermolabile compounds that are easily oxidized or hydrolyzed, so they can be affected during extraction, storage, and transport. Furthermore, after application onto plants, they volatilized quickly and start degrading, leading to short persistence and low efficacy in the field. These drawbacks can be overcome by a suitable formulation through encapsulation or nanoparticles synthesis. A product formulation is a homogeneous and stable mixture of components put together according to a specific procedure with the purpose of increasing the biological activity, stability, persistence, and efficiency, while decreasing the toxicity of the product. The selected formulation depends on the intended use and mode of application, the targeted phytopathogen, and the degradation factors present in the ecosystem [16].

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