












# EFFECTO INSECTICIDA Y REPELENTE DE ACEITES ESENCIALES OBTENIDOS DE LA FLORA AROMÁTICA ARGENTINA

## INSECTICIDAL AND REPELLENT EFFECTS OF THE ESSENTIAL OILS OBTAINED FROM ARGENTINE AROMATIC FLORA

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### SUMMARY

Global population is expected to increase to 9x10<sup>9</sup> individuals by 2050, which highlights the need to produce more food in a more sustainable way. The demand for alternatives to synthetic insecticides is reflected in the increasing amount of research dealing with essential oils as insecticidal and repellent compounds. Argentina has large regions of tropical, temperate, and cold climates, where many essential oil-producing plants grow and develop. In this context, the aim of the present study was to revise the most relevant literature about the insecticidal and repellent properties of essential oils from Argentine aromatic flora. The first section of the present review covers those essential oils used to control insects that are affect human and animal health, such as mosquitoes, flies, bed bugs, and vinchucas. The second part addresses essential oils that could be used as insecticides and repellents in horticulture and agriculture, such as moths, bugs, fruit flies, different phloem-sap-feeding insect species that attack vegetable and fruit crops, and weevils and beetles that affect stored grains and food commodities. Throughout this review, the toxicity of the most bioactive essential oils is discussed by considering their chemical profile and their major pure compounds molecular features. This literature review highlights the enormous potential of Argentine essential oils to be included in repellent and insecticidal formulations.

### KEY WORDS

Argentine aromatic plants, essential oils, insecticidal effect, repellency.

### RESUMEN

Se espera que la población mundial sea de 9x10<sup>9</sup> de habitantes para el año 2050. La demanda de alternativas al uso de insecticidas sintéticos está reflejada en la creciente cantidad de investigaciones sobre el efecto insecticida y repelente de los aceites esenciales. Argentina cuenta con grandes regiones de clima tropical, templado y frío, donde habitan muchas especies de plantas aromáticas. En este contexto, el objetivo del presente estudio fue revisar la literatura más relevante sobre las propiedades insecticidas y repelentes de los aceites esenciales de la flora aromática argentina. La primera sección de la presente revisión se enfoca en aceites esenciales que son utilizados para el control de insectos que afectan la salud humana y animal, como moscas, mosquitos y vinchucas. La segunda parte aborda los aceites esenciales que podrían usarse como insecticidas y repelentes en la horticultura y la agricultura, como polillas, moscas de la fruta, chinches y otros insectos chupadores en cultivos de oleaginosas, vegetales y frutas; también escarabajos y gorgojos que atacan granos almacenados y productos alimenticios. A lo largo de esta revisión, se analiza la toxicidad de los aceites esenciales más bioactivos considerando su perfil químico y las características moleculares de sus principales compuestos puros. Este trabajo de revisión resalta el gran potencial de los aceites esenciales obtenidos de plantas aromáticas argentinas.

### PALABRAS CLAVE

Aceites esenciales, efecto insecticida, plantas aromáticas argentinas, repelencia.


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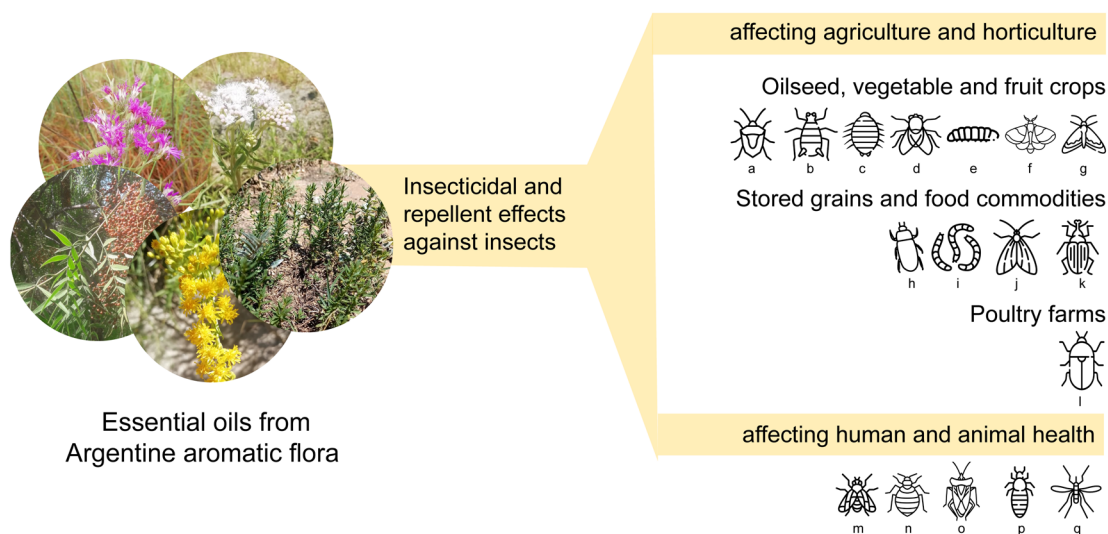
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## INTRODUCTION

The excessive use of synthetic insecticides has been associated with harmful effects on living organisms and the environment. This situation has raised a general concern in global population, leading to the development of bioactive products from natural sources. In this context, essential oils (EOs) obtained from aromatic and medicinal plants have been proposed as novel insecticides and repellents to overcome pest problems in human health, veterinary, and agricultural areas (Fig. 1; Fierascu *et al.*, 2021). Essential oils are hydrophobic mixtures of volatile organic compounds (VOCs), which are obtained from specific plants tissues and organs, such as flowers, stems, seeds, and roots. Some of the main constituents of EOs include alcohols, aldehydes, ketones, phenols, esters, ethers, monoterpenes and sesquiterpenes in varying proportions (Pandey *et al.*, 2017; Achimón *et al.*, 2021). Pharmaceutical and agrochemical industries are constantly exploring EOs or their pure VOCs to develop effective natural formulations that guarantee consumer safety and have clearly defined modes of action against insect pests (Fierascu *et al.*, 2021).

Global population is expected to increase to  $9 \times 10^9$  individuals by 2050, which highlights the need to produce more food in a more sustainable way (Marrone, 2014). For this reason, several large agrochemical companies have invested in biopesticides, promoting the continuous growth of biopesticide market (Marrone, 2014). In this context, the EOs global market is predicted to garner around USD 15 billion by 2028, with an annual growth of 15% (Inkwood Research, 2022). The main factors responsible for such progress are the ecological imbalance caused by synthetic pesticides and the increasing popularity of organic agriculture, promoted by the growing consumer demand for healthy products.

The use of biopesticides is supported by the strict regulations imposed by the United States Environmental Protection Agency (EPA) and the European Union (EU). In this context, some European countries launched programs for the reduction of synthetic pesticides and the promotion of biopesticides, such as the Ecophyto 2018 plan presented by France and Denmark (“Green Growth” program) that provides financial support for the development of alternative phytosanitary products. In Europe,



**Fig. 1.** Potential applications of Argentine EOs against different species/ groups of insects: a: *Nezara viridula*; b: Aphididae; c: *Planococcus ficus*; d: *Ceratitis capitata*; e: Caterpillars; f: *Spodoptera* sp.; g: *Plutella xylostella*; h: Beetles (*Rhizopertha dominica*, *Tribolium castaneum*, *Tenebrio molitor*); i: Beetle larvae; j: *Plodia interpunctella*; k: *Sitophilus* sp.; l: *Alphitobius diaperinus*; m: *Musca domestica*; n: *Cimex lectularius*; o: *Triatoma infestans*; p: *Pediculus humanus capitis*; q: Mosquitoes.

Netherlands, France, and Germany are the leading exporters of EOs; in America, United States, Canada, and Mexico are the major countries that make sizeable contributions to the production of EOs, followed by Argentina, Paraguay, Uruguay, Guatemala, and Haiti (Barbieri & Borsotto, 2018). However, regardless of the amount of EOs produced, it should be considered that Argentina has large regions of tropical, temperate, and cold climates. This is important since there are many phytogeographical regions (environmental factors and growing conditions) where many species of EOs-producing plant species grow and develop.

This study set out to revise the most relevant literature about the insecticidal and repellent potential of the EOs of aromatic plants from Argentina. Studies of the last 30 years obtained from the electronic databases Google Scholar, Science Direct, and Scielo were included if they met the following criteria: (1) the studies evaluated the insecticidal or repellent activity of EOs against insect species affecting humans, animals, crops, and fruits; and (2) the studies evaluated EOs extracted from plant species native to Argentina. The first section of this review will focus on the use of EOs for the control of insects that affect human and animal health, and the second section will cover those EOs used to control insect species that affect crops and fruits (Table 1).

### Cytotoxic Effects: Are EOs safe?

Before using new substances for medicinal or agricultural purposes, their potential toxicity to eukaryotic cells must be properly evaluated. The brine shrimp (*Artemia salina*) is an ideal model organism for general toxicity assays because of their wide geographical distribution, adaptability to different environmental conditions, capability to use several nutrient resources, and the high availability of eggs that can be stored for many years (Rajabi *et al.*, 2015). For these reasons, the brine shrimp is extensively used in preliminary toxicological studies that screen a large number of substances for drug discovery in medicinal plants. Many aromatic plants are widely used in traditional medicine and popular infusions; but, in general, their cytogenotoxic properties have not been evaluated. However, among the native species tested, *Aloysia polystachya* (LC<sub>50</sub> 6459.0 mg/mL), *Minthostachys*

*verticillata* (LC<sub>50</sub> 1848.0 mg/mL), *Aloysia triphylla* (LC<sub>50</sub> 1279.0 mg/mL), and *Schinus polygamus* (LC<sub>50</sub> 1179.0 mg/mL) were considered non-toxic to *A. salina*, while the EOs of *Hyptis mutabilis* (accepted name *Cantinoa mutabilis*) (LC<sub>50</sub> 30.0 mg/mL), and *Psila spartioides* (accepted name: *Pseudobaccharis spartioides*) (LC<sub>50</sub> 14.0 mg/mL) exhibited high toxicity (Oliva *et al.*, 2007). Another study that evaluated the toxic effects of EOs using human peripheral blood mononuclear cells (PBMC) and mice bone marrow cells showed that the EO of *M. verticillata* was not cytogenotoxic *in vitro* and did not induce cytotoxic and apoptotic effects in human PBMC at concentrations that ranged from 100 to 1000 µg/mL (Escobar *et al.*, 2012). Furthermore, in *in vivo* assays, *M. verticillata* EO did not increase the frequency of micronuclei in mice bone marrow cells, and the ratio of polychromatic/normochromatic erythrocytes was not modified at concentrations between 25-500 mg/kg (Escobar *et al.*, 2012). These findings would indicate that *M. verticillata* EO is a safe substance to be used as a therapeutic agent.

### EOs used to control insects that affects human and animal health

#### Ectoparasites

*Pediculus humanus capitis* (Pediculidae): The head louse is an obligate ectoparasite of humans, which is transmitted by direct host-to-host contact. This infestation is one of the most frequent among people, especially in children and adolescents. Different topical chemical insecticides are currently used for the treatment against head lice such as permethrin, allethrin, deltamethrin, and malathion. However, these insecticides tend to be harmful for children due to their underdeveloped immune system and detoxification mechanisms. An additional problem is that the repeated use of pediculicides leads to the emergence of resistance, which highlights the need for new products based on natural compounds (Yones *et al.*, 2016). One of the parameters most frequently used to compare the toxic effects of EOs on head louse adults in toxicity assays is the median knockdown time (KT<sub>50</sub>), *i.e.*, the time in minutes to knockdown of 50% of exposed insects of each experimental unit. The toxic effect of several native species against head lice was tested in Petri dishes containing 50

**Table 1.** Bioactivity of Argentine EOs against different species of insects.

Plant Family	EOs	Effect	Insect species	Reference
Amaranthaceae	<i>Dysphania ambrosioides</i>	Insecticide	<i>Alphitobius diaperinus</i>	Arena <i>et al.</i> , 2018
			<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998
			<i>Sitophilus zeamais</i>	Chu <i>et al.</i> , 2011
			<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2010
Anacardiaceae	<i>Schinus areira</i>	Repellent	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2006
		Insecticide	<i>Pediculus humanus capitis</i>	Gutiérrez <i>et al.</i> , 2016
			<i>Metopolophium dirhodum</i>	Chopa & Descamps 2012
	<i>Schinus molle</i>	Repellent	<i>Musca domestica</i>	Wimalaratne <i>et al.</i> , 1996
		Insecticide	<i>Pediculus humanus capitis</i>	Gutierrez <i>et al.</i> , 2009
			<i>Cimex lectularius</i>	Machado <i>et al.</i> , 2019
			<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998
<i>Schinus molle</i> var. <i>areira</i>	Repellent, insecticide	<i>Rhizopertha dominica</i>	Benzi <i>et al.</i> 2009	
Apiaceae	<i>Azorella cryptantha</i>	Repellent	<i>Triatoma infestans</i>	Lopez <i>et al.</i> , 2012
		Insecticide	<i>Ceratitidis capitata</i>	Lopez <i>et al.</i> , 2012
	<i>Azorella trifurcata</i>	Repellent	<i>Triatoma infestans</i>	López <i>et al.</i> , 2018
	<i>Eryngium</i> spp.	Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998
	<i>Gymnophyton polycephalum</i>	Repellent	<i>Triatoma infestans</i>	Lima <i>et al.</i> , 2011
Asteraceae	<i>Acanthostyles buniifolius</i>	Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998
	<i>Ambrosia tenuifolia</i>	Repellent	<i>Tribolium castaneum</i>	Saran <i>et al.</i> , 2019
	<i>Artemisia mendozana</i>	Repellent	<i>Triatoma infestans</i>	Lima <i>et al.</i> , 2011
	<i>Baccharis articulata</i>	Repellent	<i>Tribolium castaneum</i>	Saran <i>et al.</i> , 2019
	<i>Baccharis darwinii</i>	Repellent	<i>Triatoma infestans</i>	Kurdela <i>et al.</i> , 2012
	<i>Baccharis salicifolia</i>	Feeding deterrent	<i>Spodoptera littoralis</i>	Sosa <i>et al.</i> , 2012
	<i>Baccharis spartioides</i>	Repellent	<i>Tribolium castaneum</i>	Saran <i>et al.</i> , 2019
			<i>Aedes aegypti</i>	Gillij <i>et al.</i> , 2008
	<i>Coreopsis fasciculata</i>	Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998
	<i>Eupatorium arnotii</i>	Settling inhibition	<i>Ropalosiphum padi</i> , <i>Myzus persicae</i>	Sosa <i>et al.</i> , 2012
	<i>Eupatorium buniifolium</i>	Repellent	<i>Triatoma infestans</i>	Guerreiro <i>et al.</i> , 2018
			<i>Aedes aegypti</i>	Gleiser <i>et al.</i> , 2011
		Insecticide	<i>Trialeurodes vaporariorum</i> , <i>Tuta absoluta</i>	Umpierrez <i>et al.</i> , 2012
			<i>Triatoma infestans</i>	Guerreiro <i>et al.</i> , 2018
Settling inhibition		<i>Ropalosiphum padi</i> , <i>Myzus persicae</i>	Sosa <i>et al.</i> , 2012	

Plant Family	EOs	Effect	Insect species	Reference	
Asteraceae	<i>Eupatorium inulifolium</i>	Settling inhibition	<i>Ropalosiphum padi</i> , <i>Myzus persicae</i>	Sosa <i>et al.</i> , 2012	
	<i>Eupatorium viscidum</i>	Settling inhibition	<i>Ropalosiphum padi</i> , <i>Myzus persicae</i>	Sosa <i>et al.</i> , 2012	
	<i>Gutierrezia mandonii</i>	Insecticide, development delay	<i>Ceratitis capitata</i>	Clemente <i>et al.</i> , 2008	
	<i>Gutierrezia repens</i>	Insecticide, development delay	<i>Ceratitis capitata</i>	Clemente <i>et al.</i> , 2008	
	<i>Helianthus petiolaris</i>	Repellent	<i>Tribolium castaneum</i>	Saran <i>et al.</i> , 2019	
	<i>Senecio adenophylloides</i>	Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998	
	<i>Senecio oreophyton</i>	Repellent	<i>Triatoma infestans</i>	Lopez <i>et al.</i> , 2018	
	<i>Senecio pogonias</i>	Repellent	<i>Triatoma infestans</i>	Lopez <i>et al.</i> , 2018	
	<i>Senecio serratifolius</i>	Repellent	<i>Tribolium castaneum</i>	Saran <i>et al.</i> , 2019	
	<i>Tagetes filifolia</i>	Insecticide	<i>Tribolium castaneum</i>	Olmedo <i>et al.</i> , 2015	
	<i>Tagetes minuta</i>	Insecticide	Repellent	<i>Aedes aegypti</i>	Gillij <i>et al.</i> , 2008
			<i>Ceratitis capitata</i>	Lopez <i>et al.</i> , 2011	
			<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998	
			<i>Alphitobius diaperinus</i>	Arena <i>et al.</i> , 2018	
			<i>Brevicoryne brassicae</i>	Mullo, 2011	
	Reproduction inhibition	<i>Acyrtosiphon pisum</i> , <i>Myzus persicae</i> , <i>Aulacorthum solani</i>	Tomova <i>et al.</i> , 2005		
	<i>Tagetes pusilla</i>	Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998	
	<i>Tagetes rupestris</i>	Insecticide	<i>Ceratitis capitata</i>	López <i>et al.</i> , 2011	
	<i>Tagetes terniflora</i>	Repellent, feeding deterrent	<i>Sitophilus oryzae</i>	Stefanazzi <i>et al.</i> , 2011	
			<i>Metopolophium dirhodum</i>	Chopa & Descamps 2012	
Insecticide		<i>Sitophilus oryzae</i>	Stefanazzi <i>et al.</i> , 2011		
		<i>Plutella xylostella</i>	Descamps & Sánchez Chopa 2019		
		<i>Pediculus humanus capitis</i>	Gutiérrez <i>et al.</i> , 2009		
		<i>Tribolium castaneum</i>	Stefanazzi <i>et al.</i> , 2011		
		<i>Ceratitis capitata</i>	López <i>et al.</i> , 2011		
		<i>Brevicoryne brassicae</i>	Mullo, 2011		
Fabaceae	<i>Zuccagnia punctata</i>	Repellent	<i>Triatoma infestans</i>	López <i>et al.</i> , 2021	
Lamiaceae	<i>Hedeoma mandoniana</i>	Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998	
	<i>Hedeoma multiflora</i>	Insecticide	<i>Musca domestica</i>	Palacios <i>et al.</i> , 2009	
	<i>Lepechinia floribunda</i>	Insecticide	<i>Musca domestica</i>	Palacios <i>et al.</i> , 2009	
	<i>Lepechinia meyenii</i>	Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998	

Plant Family	EOs	Effect	Insect species	Reference
Lamiaceae	<i>Mentha pulegium</i>	Repellent	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2006
	<i>Minthostachys mollis</i>	Repellent	<i>Aedes aegypti</i>	Gillij <i>et al.</i> , 2008
		Insecticide	<i>Aedes aegypti</i>	Chantraine <i>et al.</i> , 1998
	<i>Minthostachys verticillata</i>	Insecticide	<i>Musca domestica</i>	Palacios <i>et al.</i> , 2009
			<i>Sitophilus zeamais</i>	Herrera <i>et al.</i> , 2014; Arena <i>et al.</i> , 2017
			<i>Planococcus ficus</i>	Peschiutta <i>et al.</i> , 2017
	<i>Rosmarinus officinalis</i>	Repellent	<i>Aedes aegypti</i>	Gillij <i>et al.</i> , 2008
		Insecticide	<i>Metopolophium dirhodum</i>	Sánchez Chopa & Descamps, 2012
<i>Satureja parvifolia</i>	Repellent	<i>Triatoma infestans</i>	Lima <i>et al.</i> , 2011	
<i>Thymus vulgaris</i>	Insecticide	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2010	
Lauraceae	<i>Cinnamomum porphyrium</i>	Insecticide	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2010
Myrtaceae	<i>Eugenia brejoensis</i>	Larvicide	<i>Aedes aegypti</i>	Da Silva <i>et al.</i> , 2015
	<i>Myrcianthes cisplatensis</i>	Insecticide	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2006
		Repellent	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2006
	<i>Myrcianthes pseudomato</i>	Insecticide	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2010
Poaceae	<i>Elyonorus muticus</i>	Repellent	<i>Sitophilus oryzae</i>	Stefanazzi <i>et al.</i> , 2011
			<i>Tribolium castaneum</i>	Stefanazzi <i>et al.</i> , 2011
		Feeding deterrent	<i>Sitophilus oryzae</i>	Stefanazzi <i>et al.</i> , 2011
Scrophulariaceae	<i>Buddleja mendozensi</i>	Insecticide	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2010
Verbenaceae	<i>Acantholippia riojana</i>	Repellent	<i>Pediculus humanus capitis</i>	Tolozza <i>et al.</i> , 2006
	<i>Acantholippia salsoloides</i>	Repellent	<i>Aedes aegypti</i>	Gleiser <i>et al.</i> , 2011
	<i>Acantholippia seriphioides</i>	Repellent	<i>Aedes aegypti</i>	Gillij <i>et al.</i> , 2008
	<i>Aloysia citriodora</i>	Repellent	<i>Tribolium castaneum</i> , <i>T. confusum</i>	Benzi <i>et al.</i> , 2014
			<i>Aedes aegypti</i>	Gillij <i>et al.</i> , 2008
			<i>Rhizopertha dominica</i>	Benzi <i>et al.</i> , 2009
			<i>Nezara viridula</i>	González <i>et al.</i> , 2010
	<i>Aloysia citriodora</i>	Insecticide	<i>Musca domestica</i>	Palacios <i>et al.</i> , 2009
			<i>Nezara viridula</i>	González <i>et al.</i> , 2010
			<i>Tribolium castaneum</i> , <i>T. confusum</i>	Benzi <i>et al.</i> , 2014
<i>Rhizopertha dominica</i>			Benzi <i>et al.</i> , 2009	
<i>Plutella xylostella</i>			Descamps & Sánchez Chopa, 2019	
<i>Diuraphis noxia</i>	Sánchez Chopa & Descamps, 2015			

Plant Family	EOs	Effect	Insect species	Reference
Verbenaceae	<i>Aloysia citriodora</i>	Insecticide	<i>Pediculus humanus capitis</i>	Gutiérrez et al., 2016
		Ovicide	<i>Nezara viridula</i>	Gonzalez et al., 2010
	Repellent	<i>Aloysia polystachya</i>	<i>Rhizopertha dominica</i>	Benzi et al., 2009
			<i>Nezara viridula</i>	González et al., 2010
			<i>Tribolium castaneum</i> , <i>T. confusum</i>	Benzi et al., 2014
			<i>Aedes aegypti</i>	Gleiser et al., 2011
	Insecticide	<i>Aloysia polystachya</i>	<i>Plutella xylostella</i>	Descamps & Sánchez Chopa, 2019
			<i>Alphitobius diaperinus</i>	Arena et al., 2018
			<i>Diuraphis noxia</i>	Sánchez Chopa & Descamps, 2015
			<i>Rhizopertha dominica</i>	Benzi et al., 2009
			<i>Tribolium castaneum</i> , <i>T. confusum</i>	Benzi et al., 2014
			<i>Nezara viridula</i>	González et al., 2010
	Ovicide	<i>Nezara viridula</i>	González et al., 2010	
	<i>Lippia integrifolia</i>	Repellent	<i>Triatoma infestans</i>	Lima et al., 2011
	<i>Lippia junelliana</i>	Repellent	<i>Aedes aegypti</i>	Gleiser et al., 2011
	<i>Lippia polystachya</i>	Insecticide	<i>Culex quinquefasciatus</i>	Gleiser & Zygadlo, 2007
<i>Lippia turbinata</i>	Insecticide	<i>Plodia interpunctella</i>	Corzo et al., 2020	
		<i>Culex quinquefasciatus</i>	Gleiser & Zygadlo, 2007	
	Development delay	<i>Plodia interpunctella</i>	Corzo et al., 2020	
Zygophyllaceae	<i>Bulnesia sarmientoi</i>	Repellent	<i>Lutzomyia longipalpis</i>	de Arias et al., 1992

μL of each EO (Gutiérrez et al., 2016). The most effective EO against head lice adults was *Schinus molle* (accepted name: *Lithrea molleoides*), with similar  $KT_{50}$  values of 10.8 min and 12.8 min for the EOs obtained from fruits and leaves, respectively. The species *Thymus vulgaris*, *Aloysia polystachya*, and *A. citriodora* showed lower toxicity, with higher  $KT_{50}$  values of 18.3, 20.6, and 38.3 min, respectively (Gutiérrez et al., 2016). Other researches evaluated the fumigant toxicity of certain Argentine EOs against permethrin-resistant head lice when 60 μL of each EO were added to a filter paper placed inside the Petri plate (Tolozá et al., 2006; Tolozá et al., 2010). These studies revealed a strong toxic effect of *Cinnamomum porphyrium* (accepted name: *Ocotea porphyria*), *Myrcianthes*

*cisplatensis*, and *M. pseudomato*, with  $KT_{50}$  values of 1.1, 1.3, 4.1 min, respectively. The species *C. porphyrium* is a tree from the Yungas region of Argentina, and its EO has eugenol, benzyl alcohol, and terpinen-4-ol as major VOCs. When these pure compounds were tested alone against the head lice,  $KT_{50}$  values of 60 min were obtained, indicating that synergisms between the major constituents of this EO might be responsible for the higher toxic effects of the EO compared to the sole compounds. Other EOs tested against the head lice exhibited moderate toxicity, such as *Schinus molle* (accepted name: *Lithrea molleoides*), *A. polystachya*, *Tagetes terniflora*, and *Buddleja mendozensis*, with  $KT_{50}$  values of 12.8, 23.4, 23.4, and 28.8 min, respectively (Gutiérrez et al., 2009; Tolozá et al.,

2006, Toloza *et al.*, 2010). In addition, the EOs of *M. verticillata*, *M. cisplatensis*, *Acantholippia riojana* (accepted name: *Aloysia riojana*), and *S. areira*, showed repellence activity between 20% and 50%, while the repellency of *Mentha pulegium* EO was 75%, similar to that of the positive control, piperonal (Toloza *et al.*, 2006). Slight differences in EO chemical composition may substantially affect repellency. For example, the EOs from *M. pulegium* and *M. verticillata* have the monoterpene ketones menthone and pulegone as their major components, yet the EO from *M. pulegium* was 3.4-fold more repellent than that from *M. verticillata* (Toloza *et al.*, 2006). These studies showed that EOs have the potential to be used as ingredients of shampoos with pediculicidal properties, in many cases against lice resistant to permethrin (Gonzalez Audino *et al.*, 2007).

*Cimex lectularius*: popularly known as bed bug, is a nocturnal hematophagous insect that feeds on human blood. The toxic effect of *S. molle* EO against the bed bug was evaluated through a topical bioassay by applying 1  $\mu$ L of the EO in the dorsal surface of the insect (Machado *et al.*, 2019). A dose of 125  $\mu$ g EO/bug produced 50% mortality after 7 days of exposure. The EO profile of *S. molle* consisted in 39% of monoterpenes hydrocarbons (mainly  $\alpha$ -pinene,  $\beta$ -pinene, and limonene) and 30% of oxygenated sesquiterpenes (mainly muurolol). The toxicity of the EOs of *Baccharis punctulata* and *Baccharis microdonta* were tested against an insecticide-resistant and a susceptible strain of *C. lectularius* through topical application assays. An aliquot of 1  $\mu$ L of each EO was applied in the dorsal surface of the insects at 50  $\mu$ g/bug, and mortality was registered for 7 days after treatment. None of the EOs exhibited high mortality to both strains when applied topically. The maximum insecticidal effect occurred with *B. punctulata* EO at 7 days after treatment, with 20% mortality for both strains (Budel *et al.*, 2018).

*Lutzomyia longipalpis*: this is a species of anthropophilic sandfly of Central and South America found in a wide variety of ecological conditions. Only adult females feed on mammal blood, serving as key vessels for the propagation of cutaneous and visceral leishmaniasis. The EO of *Bulnesia sarmientoi* did not show insecticidal effects, but a repellent activity of 93% at a concentration of 2.5  $\mu$ g/10 cm<sup>2</sup> of skin, higher than that of the positive

control AUTAN (commercial product composed of 33% of the active principle diethyltoluamide) that exhibited a repellent effect of 81% (de Arias *et al.*, 1992). Another study assessed the growth inhibitory activity of *B. sarmientoi* EO on promastigote forms of *Leishmania amazonensis* at concentrations ranging from 30 to 500  $\mu$ g/mL, and a strong anti-leishmanial activity was reported with an IC<sub>50</sub> of 85.6  $\mu$ g/mL, with guaiol and 2-undecanone as the prevalent components of the EO (Andrade *et al.*, 2016).

#### Disease vectors

*Mosquitoes*: adult mosquitoes are important vectors of parasitic diseases such as malaria and filariasis, and several arboviral diseases such as yellow fever, Chikungunya, West Nile, dengue fever, and Zika, responsible for important health problems in tropical and subtropical regions in the world. The mosquito life cycle consists of egg, larva, pupa, and adult stages, with the immature stages being the target of several natural and synthetic products. In this regard, many studies have been conducted using different EOs of the Argentine aromatic flora (Chantraine *et al.*, 1998). For example, EOs of *S. molle*, *Eryngium* spp., *Baccharis* spp., *Coreopsis fasciculata*, *Senecio adenophylloides* (accepted name: *Culcitium rufescens*), *Tagetes minuta*, *Tagetes pusilla* (accepted name: *Tagetes filifolia*), produced 100% mortality to 3<sup>rd</sup> stage *Aedes aegypti* larvae at a dose of 100 mg/L (Chantraine *et al.*, 1998). Furthermore, the EOs of *Acanthostyles buniifolius*, *Chenopodium ambrosioides* (accepted name: *Dysphania ambrosioides*), *Hedeoma mandoniana*, *Lepechinia meyenii*, and *Minthostachys mollis* showed slightly lower insecticidal activities, between 80-95% (Chantraine *et al.*, 1998). Another study evaluated the toxic effect of the EO extracted from *Lippia polystachya* (accepted name: *Aloysia polystachya*) and *Lippia turbinata* on 4<sup>th</sup> stage larvae and adults of *Culex quinquefasciatus* at 24 h post-treatment. At 160 ppm, the former EO produced 79.5% and 6.7% mortality, while the latter showed 90.7% and 83.8% mortality to the larvae and adult, respectively (Gleiser & Zygadlo, 2007). Although the EOs of *L. polystachya* and *L. turbinata* have the terpene ketones  $\alpha$ -thujone and carvone as their main components, *L. turbinata* was also characterized by a high concentration of  $\beta$ -caryophyllene (Gleiser & Zygadlo 2007). These



EOs were reported to induce behavioral changes in *C. quinquefasciatus* larvae at sublethal doses, such as a decrease in the ambulation speed and the total time of ambulation (Kembro *et al.*, 2009). These changes in the locomotion pattern could be attributed to the neurotoxic effect of  $\alpha$ -thujone since it acts affecting the GABA receptors of insects (Kembro *et al.*, 2009). Additionally, the EO of *Eugenia brejoensis* exhibited insecticidal activity against the 4<sup>th</sup> larval stage of *A. aegypti* with an LC<sub>50</sub> value of 214 ppm, and  $\beta$ -caryophyllene and cadinene as its major compounds (da Silva *et al.*, 2015). The larvicidal activity of  $\beta$ -caryophyllene was also reported against different species of mosquitoes belonging to the genera *Anopheles* and *Culex*, with LC<sub>50</sub> values that ranged from 41 to 48  $\mu$ g/mL (Govindarajan *et al.*, 2016).

*Mosquito repellents*: five compounds are currently used as topical insect repellents: DEET (synthetic), p-menthane-3,8-diol (PMD; natural or synthetic), hydroxy-ethyl isobutyl piperidine carboxylate (Picaridin; synthetic), ethyl 3-[acetyl (butyl) amino] propanoate (IR3535; synthetic), and N, N-diethylphenyl-acetamide (DEPA; synthetic) (Bohbot *et al.*, 2014). As consumers have become extremely health conscious, the insect repellent market has suffered significant growth over the past few years. Indeed, the mosquito repellent market is expected to generate over \$ 9,600 million by 2026 (Aniket & Roshan: <https://www.alliedmarketresearch.com/insect-repellent-market>).

The repellence of EOs of several Argentine aromatic plants against *Aedes aegypti* was evaluated by Gleiser *et al.* (2011). The laboratory evaluation of the repellent activity consisted in introducing the forearm inside a glass cage. The forearm was protected with a latex surgical glove and a paper sleeve that was previously treated with the EO. The RD<sub>50</sub> values, *i.e.* the doses at which 50% of the specimens are repelled, were estimated for *Acantholippia salsoloides* (accepted name: *Aloysia salsoloides*), *Aloysia catamarcensis*, *A. polystachya*, *Lippia integrifolia*, *Lippia junelliana*, *Baccharis salicifolia*, *Eupatorium buniifolium* (accepted name: *Acanthostyles buniifolius*), and *T. filifolia*. The most repellent EOs were *L. junelliana* with a RD<sub>50</sub> value of 0.005  $\mu$ L/cm<sup>2</sup> skin, followed by *A. salsoloides*, *A. polystachya*, and *E. buniifolium* with RD<sub>50</sub> values of 0.02  $\mu$ L/cm<sup>2</sup> skin for the three species. The major components of *L. junelliana*

EO were camphor, limonene, and  $\beta$ -myrcene. The repellent properties of these monoterpenes tested alone against *A. aegypti* and other species of mosquitoes have also been reported (Hwang *et al.*, 1985).

Other native species have been screened for their repellent potential against *A. aegypti*, such as *Achyrocline satureioides*, *Baccharis spartioides* (accepted name: *Pseudobaccharis spartioides*), *T. minuta*, *T. pusilla*, *H. mutabilis*, *M. mollis*, *Anemia tomentosa*, *Acantholippia seriphioides*, *Aloysia citrodora*, and *Rosmarinus officinalis* (accepted name: *Salvia rosmarinus*) (Gillij *et al.*, 2008). The repellency time was recorded as the time elapsed between the applications of the repellent until the test subject received a mosquito bite. At 90% EO concentration (7.6  $\mu$ L/cm<sup>2</sup> skin), *A. seriphioides*, *A. citrodora*, *B. spartioides*, *M. mollis*, *R. officinalis*, and *T. minuta* effectively repelled mosquitoes for 90 min. At 12.5% (1.06  $\mu$ L/cm<sup>2</sup> skin), the lowest concentration tested, only *B. spartioides* and *A. citrodora* still showed repellency times of 90 min. The analyses of the chemical composition of these EOs suggest that limonene and camphor were the main components responsible for the repellent effects (Gillij *et al.*, 2008).

*Triatoma infestans*: popularly called “vinchuca”, is the most important vector of the protozoan parasite *Trypanosoma cruzi*. This parasite causes the Chagas disease, which currently affects more than 5 million people in Latin America according to the Pan American Health Organization. Different EOs have been evaluated in an attempt to find natural compounds against this insect. The species *Azorella cryptantha* and *Azorella trifurcata* have been tested as repellents against fifth instar nymphs of *T. infestans*. The repellent activity assay consisted of a filter paper disk divided into two halves, one of a treated EOs and the other one untreated (control). The insect distribution was recorded at 1, 24, and 72 h after releasing the insects. *Azorella cryptantha* EO showed 76% repellency at 1 h and reached 100% of repellency at 24 h and 72 h, equal to that showed by the positive control, tetramethrin (López *et al.*, 2012). On the other hand, *A. trifurcata* exhibited 76% repellency at 24 h, with lower percentages at 1 h (López *et al.*, 2018). The main components of *A. cryptantha* were the terpene hydrocarbons  $\alpha$ -thujene,  $\alpha$ -pinene, and  $\delta$ -cadinene whereas the major compound of

*A. trifurcata* EO was the sesquiterpene alcohol spathulenol (López *et al.* 2012, López *et al.*, 2018). Other EOs that were evaluated against nymphs of *T. infestans* were *Senecio pogonias* and *Senecio oreophyton* (López *et al.*, 2018), showing repellency values of 60 and 68% at 24 h, respectively. These EOs were characterized by high amounts of the bicyclic monoterpene  $\alpha$ -pinene. At 50%, the highest concentration tested, EOs from *E. buniifolium* obtained from plants grown in different environments showed repellency values that ranged from 50% to 100%. The major constituent of *E. buniifolium* EOs was also  $\alpha$ -pinene, which was reported as an effective repellent against *T. infestans* nymphs. In addition, those nymphs submitted to this test were killed after 12 h (Guerreiro *et al.*, 2018). Regarding the fumigant toxicity test, the mortality was 100% when all EOs were tested at 50  $\mu$ L EO/L air. The high volatility of the EOs is an important factor that allows them to penetrate the holes and cracks of walls where *T. infestans* lives, reaching the insect respiratory system, and causing their death. The repellent effect of *A. seriphoides*, *Artemisia mendozana*, *Gymnophyton polycephalum*, *Satureja parvifolia* (accepted name: *Clinopodium gilliesii*), *Tagetes mendocina*, and *L. integrifolia* was also evaluated against nymphs of *T. infestans*. Other EOs that were tested as repellents using the same methodology were *G. polycephalum* and *L. integrifolia* with increasing repellence percentages from 1 h to 72 h (Lima *et al.*, 2011). The main components of the essential oil of *G. polycephalum* were hydrocarbons, mainly camphene,  $\alpha$ -phellandrene and ocimene isomers, while *L. integrifolia* was characterized by high amounts of africanone and integrifolone (Lima *et al.*, 2011). The species *A. mendozana* and *S. parvifolia* presented an opposite pattern. Both EOs showed 100% repellency at 1 h, but their bioactivity decreased over time, particularly *S. parvifolia*, that showed only 12% repellency at 72 h (Lima *et al.*, 2011). On the other hand, two *Senecio* species from Cuyo region of Argentina, *S. pogonias* and *S. oreophyton* showed lower repellent activity, with maximum values of 76% for *S. pogonias* and 68% at 1 h and 24 for *S. oreophyton*, respectively (López *et al.*, 2018). Furthermore, the EO of *Baccharis darwinii* collected in Argentine Patagonia showed a repellent activity of 76% at 1 h and raised to 100% at 24 and 72 h at a dose of

0.5%, with limonene, thymol, and 4-terpineol as its main constituents (Kurdelas *et al.*, 2012). Guerreiro *et al.* (2018) evaluated the repellent effect of *E. buniifolium* EO a two-choice bioassay, where two flasks are connected with a glass tube with a hole in the center. In these binary choice bioassays, the EO exhibited a marked repellent activity, mostly at the concentrations of 50%. The most predominant compound of this EO is S,S-(–)- $\alpha$ -pinene, followed by ocimene, limonene, and 2-carene. An evaluation of the enantiomers of  $\alpha$ -pinene showed that the repellency against *T. infestans* was higher in the (–) enantiomer of  $\alpha$ -pinene than in the (+) one (Guerreiro *et al.*, 2018). Furthermore, the authors aimed to evaluate the fumigant and topical toxicity of *E. buniifolium* EO against *T. infestans*. At a concentration of 50  $\mu$ L/L air, 100% mortality was observed, while by topical application mortality values dropped to 20% (Guerreiro *et al.*, 2018).

Failures in using natural compounds as insecticides or repellents are often related to the rapid degradation of the active agent. For this reason, the incorporation of the compounds of interest in polymeric systems enables their controlled and sustained release. Lopez *et al.* (2021) included the EO of *Zuccagnia punctata* in poly-( $\epsilon$ -caprolactone) matrices and registered the repellent effect from 1 h to 96 h. The average repellency was 89% when the EO was applied alone from 1 to 72 h, significantly higher compared to the polymeric matrix treatment, where repellence reached the maximum value of 66% within the same time frame. However, at 96 h, the repellence of the EO alone decreased significantly to 40%, while the polymeric system remained at 66%, which might be related to the lower volatilization of the EO when it is incorporated in a polymeric system (López *et al.*, 2021).

#### Mechanical vectors

*Musca domestica*: houseflies are domestic pests of great importance in public health since they can fly for several kilometers carrying a wide variety of organisms on their mouthparts, hairs, and feces. They serve as mechanical vectors to many microorganisms and parasites responsible for more than 100 human and animal gastrointestinal diseases (Palacios *et al.*, 2009).

The leaves of *S. molle* are reported to be a traditional repellent of houseflies. The EO of *S.*

*molle* was evaluated against *M. domestica* using a two-choice bioassay and showed 100% repellency at 0.8 mg/ 25  $\mu$ L of a sugar solution (Wimalaratne *et al.*, 1996). Other Argentine species that were tested as fumigant insecticides were *M. verticillata* and *Hedeoma multiflora*, and showed LC<sub>50</sub> values of 0.5 and 1.3 mg/dm<sup>3</sup>, respectively. These LC<sub>50</sub> values evidence great insecticidal properties given that the LC<sub>50</sub> value of positive control DDVP was 0.5 mg/dm<sup>3</sup> (Palacios *et al.*, 2009). These EOs are characterized by high amounts of R - (+) – pulegone and menthone, with 69% and 12% for *M. verticillata* and 52% and 24% for *H. multiflora* (Palacios *et al.*, 2009). The insecticidal bioassay using these pure compounds reported LC<sub>50</sub> values of 1.7 mg/dm<sup>3</sup> for R - (+) – pulegone and 8.6 mg/dm<sup>3</sup> for menthone. The comparison between the LC<sub>50</sub> values of the EOs and those of the major components suggests that the toxic effect on *M. domestica* could be due to synergisms between the components of the EOs. Other EOs result in moderate toxicity to *M. domestica*, such as *A. citrodora* and *Lepechinia floribunda*, requiring doses of 26.7 and 20.6 mg/dm<sup>3</sup> to induce 50% mortality (Palacios *et al.*, 2009).

**Insect pests in poultry farms:** the darkling beetle *Alphitobius diaperinus* is one of the most common pests in poultry farms worldwide. This beetle acts as a mechanical vector favoring the dispersion of viruses, fungi, and bacteria. In addition, both adults and larvae cause skin lesions on birds, inducing stress. The contact toxicity of *Dysphania ambrosioides* and *T. minuta* was tested after 24 h of exposure (Arena *et al.*, 2018). The toxicity of the EOs was higher than that of the synthetic insecticide, cypermethrin, which showed an LC<sub>50</sub> value above 900  $\mu$ g/cm<sup>2</sup>. Moreover, *D. ambrosioides* was more bioactive, with an LC<sub>50</sub> value of 17.7  $\mu$ g/cm<sup>2</sup>, almost 6 times lower than the LC<sub>50</sub> value of *T. minuta* (Arena *et al.*, 2018). Another EO tested as insecticide on *A. diaperinus* was *A. polystachya*. This species demonstrated strong insecticidal activity in both contact and fumigant toxicity assays, with LC<sub>50</sub> values of 27.3  $\mu$ L/L of air and 0.1  $\mu$ L/cm<sup>2</sup>, respectively.

### EOs as botanical insecticides and repellents in organic agriculture and horticulture

Organic agriculture is a production system that focuses on ecological principles as the basis for crop

production. The organic certification ensures that all stages of the production process are in agreement with ecological and environmental standards, allowing a farm to label and sell its products as organic. Different accredited certification agencies work successfully around the world to verify and certify organic agriculture. In the USA, the organic production standards are called United States Department of Agriculture- National Organic Program (USDA-NOP); in the European Union (EU) the organic certification process is conducted by the Ecological Certification Organization (ECOCERT), but each European country may also have its own. Even though ECOCERT is based in Europe, it conducts inspections in more than 80 countries, being one of the largest organic certification organizations in the world. In Argentina, the official organism that certifies organic agriculture is SENASA (SENASA, 2019-2020). The Advisor Committee on Bio-inputs for Agriculture Use of Argentina (in Spanish Comité Asesor en Bioinsumos de Uso Agropecuario - CABUA) was created by Resolution SAGyP 7/2013 (National Advisory Commission on Agricultural Biotechnology – in Spanish Comisión Nacional Asesora de Biotecnología Agropecuaria - CONABIA), with the aim of providing all technical information about the regulatory framework and the necessary requirements that bio-inputs must comply to be used in the agricultural sector (Mamani & Filippone, 2018). According to CABUA, a bio-input is defined as “Any biological product that consists of or has been produced by microorganisms or macroorganisms, extracts or bioactive compounds derived from them and that is intended to be applied as an input in agricultural, food, agro-industrial or agro-energy production” (Mamani & Filippone, 2018). According to this definition, EOs are considered agricultural bio-inputs.

### Oilseed, vegetable, and fruit crops

*Plutella xylostella* (Lepidoptera: Plutellidae) is one of the most important insect pests of *Brassica napus*, an oilseed with big expansion in the last few years (Descamps & Sánchez Chopa, 2019). The EOs of *A. citrodora*, *A. polystachya*, and *T. terniflora* were evaluated against larvae of *P. xylostella* through contact toxicity assays. *Aloysia polystachya* showed 77% mortality at 10% w/v after 72 h of exposure, while EOs from *A. citrodora*

and *T. terniflora* were less toxic, with 44% mortality at the same concentration (Descamps & Sánchez Chopa, 2019).

On the other hand, *Spodoptera littoralis* is a species of moth distributed worldwide, a pest of many cultivated plants and crops. The sixth instar of *S. littoralis* larvae was fed with the EOs of *B. salicifolia*, *E. buniifolium*, *E. inulifolium*, *E. arnotti* and *E. viscidum* (50 µg/larva), and changes in the larval body weight and food consumption were evaluated. Only the EO of *B. salicifolia* reduced both larval growth and feeding, evidencing post-ingestive toxicity (Sosa *et al.*, 2012). This toxicity could be caused by the presence of the terpene hydrocarbons  $\alpha$ -thujene,  $\alpha$ -phellandrene, and p-cymene in this EO. Indeed, the aromatic monoterpene p-cymene demonstrated to be a highly toxic compound to larvae of *S. littoralis*, showing LD<sub>90</sub> values < 100 µg/cm<sup>3</sup> in fumigant acute toxicity tests (Pavela, 2010). The effect of sublethal doses of *M. pulegium* EO was assessed on the fertility of *S. littoralis* 4<sup>th</sup> instar larvae by Pavela (2012). While 1.1 viable larvae were obtained in the control, the number of viable larvae obtained from those treated with *M. pulegium* EO was 41% lower. Another work reported the high fumigant toxicity of *Artemisia absinthium* EO against 3<sup>rd</sup> instar larvae of *S. littoralis*. This EO showed an LC<sub>50</sub> value of 10.6 µL/L air, with the bicyclic monoterpene ketone camphor as the major constituent (Dhen *et al.*, 2014).

The moth species *Spodoptera frugiperda* damages and destroys a wide variety of economically important crops, such as maize and cotton. Sosa *et al.* (2017) evaluated the insecticidal activity and sublethal effects of the sesquiterpenes eudesmanes isolated from *Pluchea sagittalis* against *S. frugiperda*. The antifeedant choice test consisted of a tube with artificial diet treated with eudesmanes in one extreme and an artificial diet without eudesmanes in the other (control). The isolated eudesmanes tested presented an antifeedant effect in a dose-dependent way. The control artificial diet was chosen by a higher number of larvae compared to the artificial diet treated with eudesmanes, with percentages that ranged from 50 to 72% larvae according to the eudesmane included in the diet. In addition to their antifeedant effects, some eudesmanes produced significant larval and pupal mortality against the first generation of eggs

oviposited by females fed with the eudesmane-treated diet (100 µg/g artificial diet), while other eudesmanes induced certain malformations in larvae (Sosa *et al.*, 2017).

The species *Nezara viridula* is a polyphagous bug widely distributed in tropical and subtropical regions of the world. In Argentina, it is one of the main pests that affect soybean, a very important crop to local economy that has been expanding since its introduction 50 years ago. Werdin González *et al.* (2010) evaluated the ovicidal activity, the contact and fumigant toxicities, and the repellent effects of the EO of *A. polystachya* and *A. citrodora* against this bug. The major constituents were carvone (83.5%) for *A. polystachya*, and citronellal (51%) and sabinene (22%) for *A. citrodora*. In general, these EOs reported contact and fumigant toxicity, indicating that the penetration of the toxic compounds could occur through the tegument or the respiratory system. Furthermore, both EOs showed good ovicidal effects at concentrations that ranged from 1.2 to 12.5 µg/egg when tested by topical application. The lipophilicity of the EOs may allow the penetration of the active compounds through the corion, thus affecting embryos. Additionally, it should be considered that the LC<sub>50</sub> values of *A. citrodora* and *A. polystachya* was 13.5 µg/mL and 29.9 µg/mL air, respectively. On the other hand, *A. polystachya* was more effective than *A. citrodora* in contact toxicity assays, with LC<sub>50</sub> values of 3.4 µg/cm<sup>2</sup> for the former and 8.1 µg/cm<sup>2</sup> for the latter, evidencing that certain EOs pure components exert their toxic effect more efficiently when entering the insect body by inhalation or by contact (Achimón *et al.*, 2022). Furthermore, both *Aloysia* species were repellent to the nymphal stage at concentrations of 5.3 and 2.6 µg/mL (Werdin González *et al.*, 2010).

*Ceratitis capitata*, commonly known as the Mediterranean fruit fly, is one of the most destructive pests of the world since it attacks different fruit crops, such as apple, pear, grapevine, orange, and plum. The topical application of *A. cryptantha* EO showed a LD<sub>50</sub> of 2.6 µg/insect for males and 9.5 µg/insect for females, at 72 h after treatment. These are encouraging results since LD<sub>50</sub> values were not statistically different from those of the positive control, cypermethrin (López *et al.*, 2012). The bioactivity of this EO can be attributed to the sesquiterpenes  $\delta$ -cadinene,  $\delta$ -cadinol, and  $\tau$ -muurolol, which were reported as

good insecticides to this pest (El-Shazly & Hussein, 2004).

The EOs of different species of *Tagetes* were evaluated against *C. capitata* in topical application assays (López *et al.*, 2011). The species evaluated were *T. minuta*, *T. rupestris*, and *T. terniflora*. These species have several monoterpene ketones as their major components, such as cis-tagetone, trans-tagetone, dihydrotagetone. At a dose of 10 µg/insect, between 20 and 35% of males and between 24 and 48% of females died after 24 h of application. A dose of 100 µg/insect caused between 85 and 90% mortality with no difference between males and females (López *et al.*, 2011). On the other hand, the olfactory activity of EOs against *C. capitata* adults was tested in a Y-tube olfactometer. The EOs of *T. minuta* and *T. terniflora* triggered an attractive response on *C. capitata*, probably due to the presence of the monoterpene hydrocarbons limonene and p-cymene (López *et al.*, 2011).

Other aromatic species tested against *C. capitata* were *Gutierrezia mandonii* and *Gutierrezia repens*, which grow in the northwestern Argentina at altitudes above 1000 meters above sea level. The EOs of these species are characterized by high concentrations of monoterpene and sesquiterpene hydrocarbons (Clemente *et al.*, 2008). Essential oils were incorporated into an artificial diet to feed the larvae, and mortality until adult emergence was recorded. The EO of *G. mandonii* and *G. repens* produced 43% and 60% mortality to *C. capitata*, respectively. Additionally, the required concentration of the EOs to avoid development in 50% of *C. capitata* larvae was 1138 ppm for *G. mandonii* and 248 ppm for *G. repens* (Clemente *et al.*, 2008).

Several investigations have shown the presence of eudesmane-type sesquiterpenoids in different genus of the Asteraceae family. Different eudesmans isolated from *P. sagittalis* showed an oviposition deterrence of 87% in *C. capitata* at a concentration of 30 µg/cm<sup>2</sup> of artificial diet. Furthermore, significant larval and pupal mortality against the first generation larvae of viable eggs oviposited by females fed with the treated diet was also observed (Sosa *et al.*, 2017).

*Planococcus ficus* (*Pseudococcidae*): commonly known as vine mealybug, this is one of the main pests of vineyards in tropical and subtropical regions

of the world. The EO of *M. verticillata* and its major components were evaluated on *P. ficus*. The results revealed that *M. verticillata* was good insecticide with 60-80% mortality at a concentration of 600 µL/L. Regarding pure compounds, the α,β-unsaturated ketone pulegone showed an LC<sub>50</sub> value of 39.6 µL/L, more toxic than menthofuran, the oxidation product of pulegone, that showed LC<sub>50</sub> value of 63.9 µL/L. In addition, the monoterpene epoxide 1,8-cineole had higher insecticidal effect than its isomer 1,4-cineole (Peschiutta *et al.*, 2017).

#### *Phloem-sap-feeding insects*

Phloem-feeding insects suck the sap from plant leaves, being considered important pests of several plant and crop species. Tomato crops are usually affected by many species, with *Trialeurodes vaporariorum* and *Tuta absoluta* being the ones of greatest incidence. The insecticidal effect of *E. buniifolium* EO was tested against *T. vaporariorum* in contact and fumigant toxicity assays. A nearly complete mortality (LD<sub>99</sub>) of *T. vaporariorum* was obtained with 0.3 mg/cm<sup>3</sup> of *E. buniifolium* EO in fumigant assays and with 0.1 mg/cm<sup>2</sup> in direct contact tests. On the other hand, the LD<sub>99</sub> value of *E. buniifolium* against *T. absoluta* was 1.5 mg/cm<sup>2</sup> in contact toxicity assays (Umpiérrez *et al.*, 2012).

Other plant species were evaluated against the aphids *Rhopalosiphum padi* and *Myzus persicae*, and percent settling inhibition (% SI) was calculated by comparing the percent of aphids present on surfaces treated with the EOs and the percent of aphids present on control surfaces. The aphid species *R. padi* and *M. persicae* were affected differently by the EOs tested, with *R. padi* being less sensitive. The species *E. buniifolium* exhibited 65% SI of *R. padi* at 10 µg/µL, with significant lower values for *E. inulifolium*, *E. arnotii*, and *E. viscidum*. On the other hand, *M. persicae* responded strongly to all the EOs tested with % SI values that ranged from 66 to 83% at 10 µg/µL (Sosa *et al.*, 2012).

In Argentina, the species of aphids *Metopolophium dirhodum* and *Diuraphis noxia* are abundant in semi-arid regions and attack crops such as wheat, barley, rye and oats, causing yield losses of 27-30% (Sánchez Chopa & Descamps, 2012). The EOs from *T. terniflora*, *R. officinalis*, and *S. areira* (leaves and fruits) were tested in contact toxicity assays against apterous and alate adults of *M. dirhodum*. The LC<sub>50</sub> of apterous adults

calculated at 24 h after exposure were 76.2 mg/mL for *T. terniflora*, 15.2 mg/mL for *R. officinalis*, 58.3 mg/mL for *S. areira* (leaves), and 76.2 mg/mL for *S. areira* (fruits). The alate forms showed statistically lower LC<sub>50</sub> values: 20.2 mg/mL for *T. terniflora*, 23.7 mg/mL for *R. officinalis*, 7.5 mg/mL for *S. areira* (leaves), and 10.5 mg/mL for *S. areira* (fruits). Additionally, all the EOs produced some degrees of repellency in adults and sublethal effects on the reproduction, development, longevity, survivorship, and fecundity, which are important parameters to achieve an effective aphid management (Sánchez Chopa & Descamps, 2012). *Diuraphis noxia* is one of the main aphid pest of wheat in the semiarid Pampas of Argentina. Essential oils from leaves of *A. polystachya* and *A. citrodora* were used against *D. noxia* in contact toxicity tests, with *A. polystachya* EO being more toxic (LC<sub>50</sub> = 7.4 mg/mL) to *D. noxia* than the EO of *A. citrodora* at 24 h after exposure (LC<sub>50</sub> = 23.7 mg/mL) (Sánchez Chopa & Descamps, 2015).

*Brevicoryne brassicae*, commonly known as the cabbage aphid, is a destructive aphid found many regions of the world. This species feeds on many members of the genus *Brassica*, especially broccoli. The EOs of *T. terniflora* and *T. minuta* were tested against *B. brassicae* adults. Pieces of broccoli of 25 cm<sup>2</sup> were submerged in different concentrations of EOs: 0.2, 0.4, 0.6, 0.8, and 1.0%, and the mortality was evaluated after 24 h of exposure. The results showed that both *Tagetes* species were effective at 24 h, with 100% of mortality at 1% (Mullo, 2011). In addition, the EO of *T. minuta* was evaluated on the reproduction of three aphid species: *Acyrtosiphon pisum* (pea aphid), *Myzus persicae* (peach-potato aphid), and *Aulacorthum solani* (glasshouse and potato aphid). The EO significantly reduced aphid reproduction, and the effect depended on EO concentration and the species of aphid involved. At the highest dose tested (1 µL/Petri plate), 100, 94, and 85% decrease in offspring number was achieved after 5 days of exposure for *A. pisum*, *M. persicae*, and *A. solani*, respectively. Furthermore, the EO was fractionated by vacuum distillation, and three fractions were obtained and analyzed by GC-MS. The fraction characterized by a high content of oxygenated monoterpenes was more effective in restricting aphid population growth, showing 95% fewer offspring at day 3 and no live aphids at day 4 (Tomova *et al.*, 2005).

## Insects that affect stored grains and food commodities

The weevils *Sitophilus zeamais*, *Sitophilus oryzae*, *Sitophilus granarium*, and *Rhyzopertha dominica*, and the moth *Plodia interpunctella* are considered to be primary pests of different cereal grains worldwide. These species cause significant damage to harvested stored grains, drastically decreasing crop yields. The attack of primary pests may facilitate the establishment of secondary pests. The difference between them is that the former have the ability to attack whole, dry, unbroken grains while the latter attack damage grains, dust, and milled products. Some of the most common secondary pest to cereal grains are the weevils *Tribolium confusum* and *T. castaneum*. Adults of *S. zeamais* were treated with EOs from *Aphylocladus decussatus* (accepted name: *Famatinanthus decussatus*), *A. polystachya*, *M. verticillata*, and *T. minuta* in fumigant toxicity assays (Herrera *et al.*, 2014). *Minthostachys verticillata* was the most toxic EO with an LC<sub>50</sub> of 116.6 µL/L. The major components of this EO were pulegone and carvone, which showed LC<sub>50</sub> values of 11.8 and 85.5 µL/L when tested alone (Herrera *et al.*, 2014). As it was mentioned before, the activity of an EO is usually attributable to its main constituents. However, the insecticidal effect of an EO is not strictly correlated with major components because the presence of minor constituents can lead to synergistic or antagonistic effects. For these reasons, the application of binary mixtures of EOs is a common strategy for pest control. In this context, Arena *et al.* (2017) assessed the fumigant toxicity of binary combinations of *M. verticillata* and *A. citrodora* EOs and obtained an LC<sub>50</sub> value of 77.6 µL/L, while the LC<sub>50</sub> value of *A. citrodora* was higher than 600.0 µL/L. Another study evaluated the fumigant toxicity of *C. ambrosioides* EO and its major constituents, ascaridole and isoascaridole, against *S. zeamais* (Chu *et al.*, 2011). The LC<sub>50</sub> values were 3.1 mg/L for the EO, 0.8 mg/L for ascaridole, and 2.5 mg/L for isoascaridole. As it can be seen, ascaridole showed three times more activity than the crude EO and isoascaridole. Ascaridole is a monoterpene with a peroxy group across position 1 to 4, which could be the responsible for its bioactivity since isoascaridole is a very similar compound but lacks the internal 1,4-peroxide. The

fumigant activity of ascaridole is comparable to that of methyl bromide, one of the currently used grains fumigants. Another study evaluated the fumigant toxicity, antifeedant effect, and repellency of *Elyonorus muticus*, *Cymbopogon citratus*, and *T. terniflora* EOs against *S. oryzae* adults. Only the EO of *T. terniflora* demonstrated moderate fumigant toxicity to *S. oryzae*, with an LC<sub>50</sub> value of 322.6 µg/cm<sup>2</sup>. Moreover, the EOs were repellent to *S. oryzae* adults with an overall repellency in the range 73-89% at 20 g/L. Regarding the antifeedant activity, the EOs had strong feeding deterrent effect, reducing the relative growth rate in *S. oryzae* adults (Stefanazzi *et al.*, 2011).

Essential oils obtained from *A. polystachya*, *A. citrodora*, and *S. molle* var. *areira* (accepted name: *Lithrea molleoides*) were tested against *Rhizopertha dominica* adults in contact, fumigant, and repellence bioassays (Benzi *et al.*, 2009). In contact toxicity bioassays, the EOs from leaves of *A. polystachya* and *S. molle* exhibited strong effect against adults of *R. dominica*, with LD<sub>50</sub> values of 0.9 and 0.6 mg/cm<sup>2</sup>, respectively. In fumigant toxicity tests, the LC<sub>50</sub> value was 0.2 mg/cm<sup>2</sup> for both *A. polystachya* and *A. citrodora* EOs, while the EO from *S. molle* showed lower toxicity with LC<sub>50</sub> of 0.6 mg/cm<sup>2</sup>. Additionally, *A. citrodora* showed 80% of repellency at the highest concentrations, almost two times higher than the other EOs tested (Benzi *et al.*, 2009).

The moth *Plodia interpunctella* is a major economic insect pest of stored products and processed food commodities found worldwide. Corzo *et al.* (2020) evaluated the insecticidal activity of *L. turbinata* EO in *P. interpunctella* third-instar larvae. The EO caused mortality in larvae in a dose-dependent manner, with an LC<sub>50</sub> value of 432.9 mg/L. Furthermore, the EO caused a delay in the pupation day in the surviving larvae, which was correlated with a low expression of the neuropeptides responsible for regulating the postembryonic development in lepidopterans (Corzo *et al.*, 2020).

The flour beetles *Tribolium castaneum* and *T. confusum* have been reported as serious secondary pests in Argentina. The EOs of *A. polystachya* and *A. citrodora* were tested as insecticides and repellents against flour beetles (Benzi *et al.*, 2014). Both EOs showed fumigant toxicity only against *T. confusum*, with LC<sub>50</sub> values of 5.9 and 5.5 mg/L air for *A. polystachya* and *A. citrodora*, respectively.

On the contrary, both EOs were toxic only to *T. castaneum* in contact toxicity assays, with the EO of *A. polystachya* being more effective (LD<sub>50</sub> = 7.4 µg/insect) than the EO of *A. citrodora* (LD<sub>50</sub> = 13.8 µg/insect). On the other hand, repellent activity was stronger with *A. citrodora*, with mean repellency values over 70% for both species, probably due to the presence of citronellal, a natural compound commonly used in commercial insect repellents (Benzi *et al.*, 2014). Another study evaluated the repellent activity of five species belonging to the family Asteraceae: *Ambrosia tenuifolia*, *Baccharis articulata*, *B. spartioides*, *Helianthus petiolaris*, and *Senecio serratifolius* (accepted name: *Culcitium serratifolium*) (Saran *et al.*, 2019). All the tested EOs exhibited repellent effect against *T. castaneum* in a dose-dependent manner, with those from *B. spartioides* and *H. petiolaris* being the most effective, showing values over 95% of repellency. The repellent activity of both EOs was improved when they were included in binary mixtures with Lemon EO, evidencing synergisms among the pure compounds of the different EOs (Saran *et al.*, 2019). Olmedo *et al.* (2015) assessed the fumigant toxicity of the EO from *T. filifolia* and its main compounds, anethole and estragole, against *T. castaneum*. The EO and anethole were the most toxic at 24 h, with CL<sub>50</sub> values of 2.4 and 2.6 µL/mL water, respectively. Additional experiments demonstrated that the toxic effect may be due to the inhibition of acetylcholinesterase activity (Olmedo *et al.*, 2015). The species *T. terniflora* showed moderate fumigant and contact toxicities to *T. castaneum* with LC<sub>50</sub> values of 362.8 µg/cm<sup>2</sup> and 217.3 µg/cm<sup>2</sup>, respectively (Stefanazzi *et al.*, 2011). Furthermore, the EO produced a repellent effect that was concentration-dependent with values of approximately 90% in both larvae and adults at the highest doses tested. The EO from *E. muticus* produced an even higher repellent effect, with 100% repellency at 40 g/L in larvae and 96% at 20 mg/L in *T. castaneum* adults (Stefanazzi *et al.*, 2011).

Mealworms are the larval stage of the mealworm beetle, *Tenebrio molitor*, a stored grain pest. Different sesquiterpenes were isolated from plants of *Tessaria absinthioides* growing in the Cuyo region, and their contact toxicity, growth alteration effects, and repellent activities were tested (García *et al.*, 2003). The compounds tessaric acid, ilicic aldehyde, costic aldehyde,

and  $\gamma$ -costic acid increased pupal stage duration along with morphological abnormalities. None of the tested compounds produced a significant mortality on larvae within the first 3 days of the experiment. Regarding repellency, ilicid aldehyde and  $\gamma$ -costic acid showed the strongest effect, with mean repellency values from 86 to 93% after 30 min of exposure at the highest concentration (80  $\mu\text{g}/\text{cm}^2$ ) (García *et al.*, 2003).

## CONCLUSIONS

Currently, the control of insect pests relies heavily on synthetic insecticides. Despite the efficacy of these chemical substances, they are associated with hazardous effects on living organisms and the environment and can lead to the development of resistance. In this context, the application of natural compounds is among the most recommended management practices to overcome these problems. The present review has examined the insecticidal and repellent activities of the EOs of many plant species native to Argentine flora, showing very encouraging outcomes. In general, the EOs more frequently evaluated were those belonging to the families Asteraceae, Lamiaceae, and Verbenaceae. Within Asteraceae, the species *E. buniifolium* and *T. minuta* demonstrated to be the most effective EOs against several species of insects; within Lamiaceae, *R. officinalis* and *M. verticillata* were the most bioactive EOs; and within Verbenaceae, *A. citrodora* and *A. polystachya* proved to be the most toxic species. In several cases, the bioactivity of the EOs was comparable or even better than that showed by the synthetic insecticides that were used as positive controls. This work highlights the enormous potential of EOs to be included in repellent and insecticidal formulations.

## AUTHOR CONTRIBUTION STATEMENTS

MPZ and JAZ: Conceptualization; FA, MB, VDB, MLP, JMH, CM, and RPP: literature research; FA, MB, VDB: writing—original draft preparation. FA, MPZ and JAZ: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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