From plant scent defense to biopesticide discovery: Evaluation of toxicity and acetylcholinesterase docking properties for Lamiaceae monoterpenes

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ABSTRACT

Monoterpenes are a highly diverse group of chemical scents that originate from plant secondary metabolic processes, one purpose of which is to serve as a defense against herbivores. Plant-derived monoterpenes produced from Lamiaceae species have demonstrated acaricidal activity against pest mites and ticks. However, the mechanism by which these compounds carry out the demise of this group of arachnids is poorly understood. Here, we review the chemistry and bioactivity of these promising compounds and subsequently assess their potential toxicity to mites and ticks through measurement of their docking ability to amino acid residues of the binding pocket of *Drosophila melanogaster* acetylcholinesterase (AChE). We identified 27 monoterpenes of the Lamiaceae family from the literature that were effective against mite (Varroidae, Tetranychidae, Eriophyidae) and tick (Ixodidae) species that are problematic in agricultural production. Screening of these compounds showed that monoterpenoids possessing methyl groups, such as carvacrol, linalool, α-terpineol, bornyl acetate, and terpine-4-ol, strongly bind to *D. melanogaster* AChE. Linalool, which fits into the binding pocket in the amino acid catalytic triad of AChE (oxyanion hole residues, hydrogen bond interaction with GLU 237, and anionic binding with TRP 83), was identified as the most promising target for further optimization studies. We propose that monoterpenes which interact strongly with amino acid residues of the AChE receptor be targeted for development of effective, naturally produced biocontrol pesticides, as this model demonstrates potential for discovery of new acaricide compounds in a high throughput manner.

1. Introduction

Naturally derived compounds have been proposed as safe, ecologically-sound and sustainable alternatives to synthetic commercial pesticides in agricultural pest management programs (Mfarrej and Rara, 2019). Plant secondary metabolites have been extensively investigated for this purpose (Hannour et al., 2018; Murcia-Meseguer et al., 2018; Djebir et al., 2019; Lukwa et al., 2018; Hu et al., 2019; El-Zemity et al., 2009), as they exhibit an array of biological activities which negatively affect arthropod pests. The globally distributed Lamiaceae family comprises approximately 7000 flowering shrubs and trees identified across 236 genera. Plants belonging to this family are characterized by the synthesis of large amounts of volatile terpenes that are stored in glandular trichomes-scent-rich organs which substantiate the

characterization of these plants as "aromatic." Essential oils (EOs) extracted from Lamiaceae such as *Mentha*, *Lavandula*, *Ocimum,* and *Rosmarinus* are valued for their fragrance and flavor, with application as culinary flavorings as well as in industrial products, such as pharmaceuticals, cosmetics, and perfumes (Singh and Pandey, 2018; Despinasse et al., 2017; Pravuschi et al., 2010; Vilanova et al., 2018; Cobellis et al., 2016).

Among plant secondary metabolites targeted for biopesticide development, monoterpenes have garnered much interest due to their demonstrated effectiveness against a wide range of arthropod pests (Regnault-Roger et al., 2012; Novato et al., 2018; Tabari et al., 2017; Lima de Souza et al., 2019). Monoterpenes contain ten carbon atoms in their chemical structure which can be assembled into a diversity of scaffolds by terpene synthases (Fig. 1) (Leferink et al., 2016, 2019). Scaffolds can be linear or ring-based (monocyclic or bicyclic) and may

include oxygen. Biosynthesis occurs by condensation of two five-carbon (C5) isoprene units in two distinct metabolic pathways known as the mevalonate and the methylerythritol phosphate pathways, producing dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), respectively, as intermediates (Mahmoud et al., 2021; Barreto et al., 2021). The head-tail condensation of these active units produces geranyl diphosphate (GPP) which is built upon to create the final monoterpene structure, catalyzed by different terpene synthases.

Monoterpenes are ubiquitous in plants and have proven insecticidal (El-Minshawy et al., 2018), acaricidal (Novato et al., 2015, 2018; Lima de Souza et al., 2019; Pereira Junior et al., 2020), molluscicidal, nematicidal, amebicidal, antibacterial (Joshi et al., 2019), antifungal (Liu et al., 2020), leishmanicidal (Dutra et al., 2016), allelopathic (Verma et al., 2018), antidepressive, antidiabetic (Deng et al., 2013; Dong and Chen, 2013), and antioxidant properties (Noacco et al., 2018;
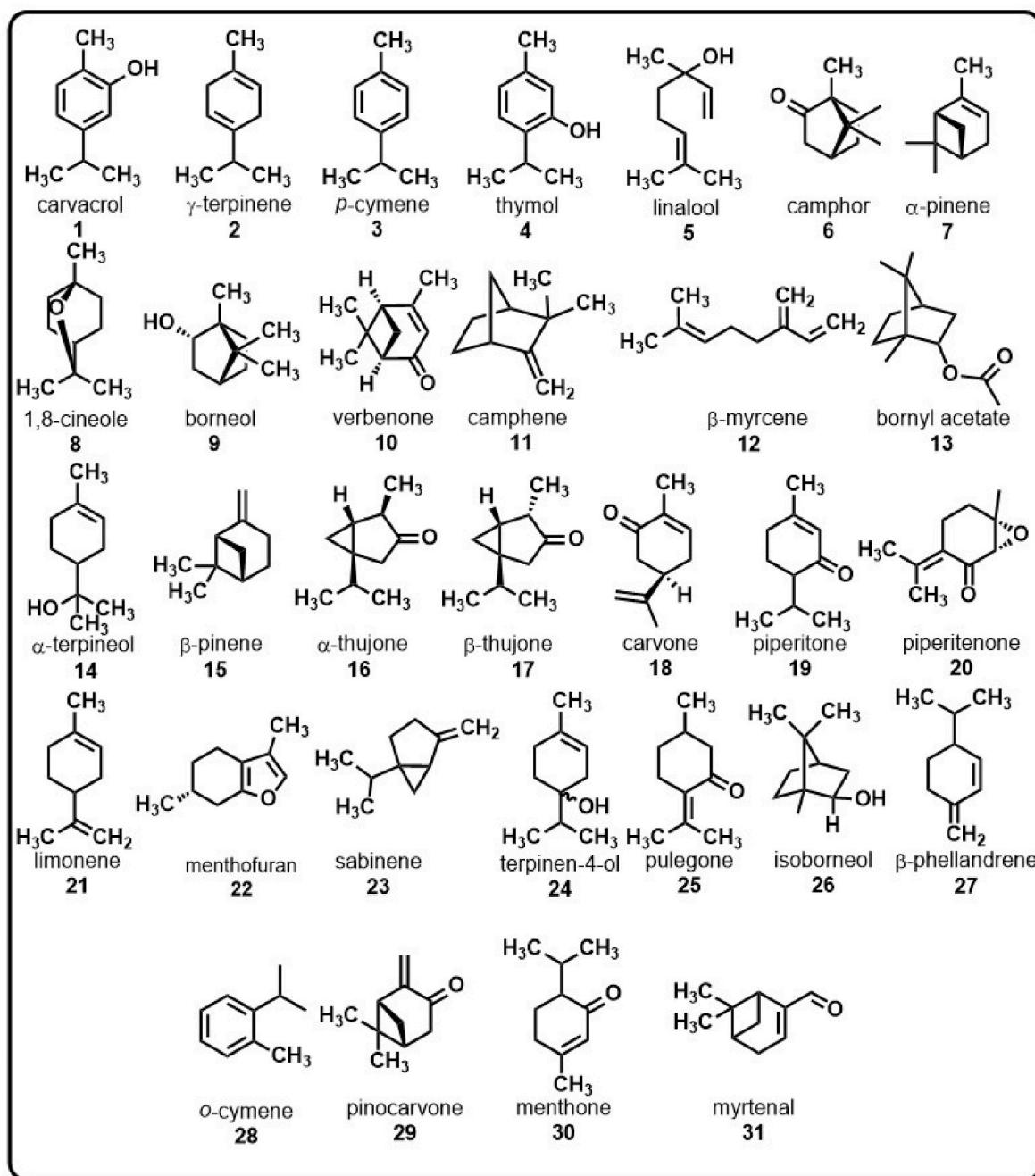

Fig. 1. Monoterpenes found in plants belonging to the Lamiaceae family.

Popović-Djordjević et al., 2019). Moreover, this class of terpenes is rapidly decomposed in the environment and exhibits minimal toxicity to mammals (Regnault-Roger et al., 2012; Vojoudi et al., 2014; Castellanos González, 2017; Chen and Dai, 2017; Van Leeuwen et al., 2015). EOs from the family Lamiaceae have been investigated as a source of natural pesticides against insects in both agricultural and veterinary realms. The large variation in the chemical composition of Lamiaceae EOs with demonstrated acaricidal activity stem from abiotic factors such as soil nutrition, water availability, temperature and physiological conditions (Djebir et al., 2019; Figueiredo et al., 2008; Attia et al., 2012; Martinez-Velazquez et al., 2011; Laborda et al., 2013; Zandi-Sohani and Ramezani, 2015; Yorulmaz Salman et al., 2014).

Within the subclass Acari, mites and ticks are responsible for significant financial losses in crop and livestock production, for which there are no truly effective countermeasures that don't have environmental consequences. Species found within the *Tetranychidae* family include several key agricultural pests such as the two-spotted spider mite *Tetranychus urticae* (Koch) and the carmine spider mite *T. cinnabarinus* (Boisd.) which are polyphagous pests, able to adapt their food source to various horticultural and ornamental plant species with a global distriubution (Helle and Sabelis, 1985; Jongejan and Uilenberg, 2004). Under the Eriophyidae family, the genera *Aceria*, *Aculus* and *Aculops* contain organisms that are devastating to agricultural production, in particular the coconut mite *Aceria guerreronis* (Keifer) which is a major nuisance in coconut groves of Africa and Asia (Navia et al., 2013). In addition, the Varroidae family contains the external parasite *Varroa destructor* (Anderson & Trueman) (*Varroa* mite) which inflicts severe losses to beehives in many countries (Ruffinengo et al., 2014). As for ticks, the Ixodidae family contains several ectoparasites of livestock that may also be a vector of secondary pathologic disease to animals and humans alike (Jongejan and Uilenberg, 2004). The cattle tick *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) is considered to be the most important tick parasite of livestock in the world (Spickler and Rovid, 2007) and is widely distributed in South and Central America as well as in Africa. In Brazil, it is estimated that 80% of the national cattle herd is infested, causing an economic loss of $3 billion dollars a year (Sato et al., 2020). Effects from infestation of *R. microplus* include decreased production and transmision of babesiosis and anaplasmosis (Spickler and Rovid, 2007).

Drosophila melanogaster (Meigen) has been a model experimental organism for decades, used as a proxy to study a variety of parameters covering a range of species, including humans (Bier, 2005). Acethylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine to acetic acid and choline which, when inhibited, paralyzes cholinergic transmission in the mite nervous system, causing their death (Ribeiro et al., 2012; Silva et al., 2004; Bu et al., 2015). Previous studies on the tick *R. microplus* have shown that esterases, particularly carboxylesterases and AChEs, are associated with acaricidal resistance (Baffi et al., 2008). Therefore, prediction of compounds that block AChE regulation and alter metabolic detoxification in mites and ticks through molecular modeling have the potential to provide a high throughput tool for identification then development of effective acaricide compounds.

Computer-based molecular docking accelerates drug discovery as it enables the evaluation of ligand libraries for shape and energetic compatibility with a known crystal structure of a receptor virtually before experimental *in vitro* validation. The virtual screening of compound libraries by molecular docking selects only the best fitting molecules for synthesis and testing, reducing the costs associated with wet-lab assays to identify lead compounds (Shoichet, 2004; Lyu et al., 2019). The discovery of chemical matter (e.g., tetraphenylporphinesulfonate, tacrine-squaramide derivatives) with inhibitory activity against AChE are examples of success that attest to the application of molecular docking in the development of novel anti-cholinesterase inhibitors (Hai et al., 2013; Svobodova et al., 2019).

Due to the large number of studies that exist which demonstrate effectiveness of natural and synthetic pesticides against species in the

phylum Arthropoda, we focused on monoterpenoids as a chemical class from the family Lamiaceae and the lesser-known mite and tick pests found within the subclass Acari. In this study, we specifically (*i*) reviewed literature which examined bioactive monoterpenes from Lamiaceae EOs against arthropod pests of agricultural and veterinary relevance and (*ii*) assessed their suitability as potential inhibitors of fruit fly *D. melanogaster* AChE through modeling and characterization of molecular docking.

2. Review of monoterpene plant sources and their acaricidal activity

2.1. Origanum

A total of six species from the genus *Origanum* have been reported to contain monoterpenoids which are active against mites and ticks (Koc et al., 2013; Cetin et al., 2009; Ramzi et al., 2017; Sertkaya et al., 2010; Çalmaşur et al., 2006) (Table 1). The aromatic alcohol carvacrol (Compound 1, Fig. 1) is the primary component of EOs extracted from *Origanum* species with concentrations varying from 18 to 93%; species reviewed here were *Origanum bilgeri* (P.H. Davis), *Origanum minutiflorum* (O. Schwarz and P.H. Davis), *Origanum elongatum* (Bonnet) (Emb. & Maire), *Origanum onites* L., *Origanum vulgare* L. and *Origanum floribudum* (Munby). Their volatiles were active against the ticks *Rhipicephalus turanicus* (Pomerantsev)*, Amblyomma americanum* L., *Hyalomma scupense* (Schulze) and *Haemaphysalis longicornis* (Acari: Ixodidae) and the mites *V. destructor*, *T. cinnabarinus* and *Psoroptes cuniculi* (Delafond) (Acari: Psoroptidae) (Table 1) (Koc et al., 2013; Cetin et al., 2009; Ramzi et al., 2017; Sertkaya et al., 2010; Carroll et al., 2017; Shang et al., 2016; Qiao et al., 2021). Apart from carvacrol, the EO components from *Origanum* species included *γ*-terpinene (Compound 2, Fig. 1) and *p*-cymene (Compound 3, Fig. 1) from *O. elongatum* as minor components; *p*-cymene (Compound 4, Fig. 1), *γ*-terpinene, linalool (Compound 5, Fig. 1), and thymol in *O. onites*; and thymol in *O. vulgare*.

2.2. Rosmarinus and Salvia

Camphor was present in species from both the *Rosmarinus* and *Salvia* genera. According to Salman et al. (Yorulmaz Salman et al., 2014), this monoterpene is the main component in *R. officinalis* EO, accounting for 35.0% of its composition, while it makes up 11.0–29.0% in *S. officinalis* extracts (Laborda et al., 2013; Yorulmaz Salman et al., 2014) (Table 1). Other compounds common to both of these genera are: *ß*-myrcene present in the EO of *R. officinalis* (9.0%) and *S. officinalis* (1.4%) (Laborda et al., 2013), *α*-pinene (Zandi-Sohani and Ramezani, 2015) and camphene (Zandi-Sohani and Ramezani, 2015). On the other hand, *α*-terpineole (Compound 14, Fig. 1), verbenone (Compound 10, Fig. 1) (Martinez-Velazquez et al., 2011; Miresmailli et al., 2006), and bornyl acetate (Compound 13, Fig. 1) were only found in *R. officinalis* (Laborda et al., 2013), while *ß*-thujone has only been detected in *S. officinalis* (Laborda et al., 2013).

Six studies have addressed the acaricidal activity from EOs of *Rosmarinus officinalis* L. (Djebir et al., 2019) Variation was present in both the identified compounds and their respective percentage in the EO. Camphor (Compound 6, Fig. 1), *α*-pinene (Compound 7, Fig. 1) and 1, 8-cineole (Compound 8, Fig. 1), and borneol (Compound 9, Fig. 1) were the main components extracted from the aerial parts of *R. officinalis* (Table 1). The EO of *R. officinalis* also contains significant amounts of 1, 8-cineole (14.0–26.0%) (Djebir et al., 2019; Martinez-Velazquez et al., 2011; Laborda et al., 2013; Yorulmaz Salman et al., 2014) as well as borneol (Compound 9, Fig. 1) (21.0–22.0%) (Zandi-Sohani and Ramezani, 2015; Salman et al., 2015). *R. officinalis* also produces minor amounts of verbenone, camphene (Compound 11, Fig. 1), *ß*-myrcene (Compound 12, Fig. 1), bornyl acetate, *α*-terpineol (Compound 14, Fig. 1), *p*-cymene, and *ß*-pinene (Compound 15, Fig. 1) (Table 1). The EOs from *R. officinalis* were active against the spider mites *T. urticae* and

Table 1

Monoterpenes identified from Lamiaceae species with demonstrated acaricidal activity.

Lamiaceae species	Major compounds[a]	Mite/tick species	Biological activities	Plants EO containing candidate insecticides[b]	Reference(s)
Origanum bilgeri	carvacrol [1] (93.0%)	*Rhipicephalus turanicus* (Acari: Ixodidae)	EO at 0.8% caused mortality in 83% of the adults within 48h. Carvacrol at 0.4% caused mortality in 63% of the adults within 120 min.	X	Koc et al. (2013)
Origanum minutiflorum	carvacrol [1] (85.0%)	*R.turanicus*	10 µl/L of the EO caused mortality in 100% of the adults within 120 min.	X	Cetin et al. (2009)
[c]*Origanum elongatum*	carvacrol [1] (67.0%–81.0%) γ-terpinene [2] (3.0%–10.0%) *p*-cymene [3] (3.0%–7.0%)	*Varroa destructor* (Acari: Varroidae)	Not reported	X	Ramzi et al. (2017)
Origanum onites L	carvacrol [1] (68.0%) *p*-cymene [3] (10.0%) γ-terpinene [2] (6.0%)	*Tetranychus cinnabarinus* (Acari: Tetranychidae)	LC_{50} of the EO = 0.69 µg/mL. LC_{90} of the EO = 3.14 µg/mL.	X	Sertkaya et al. (2010)
	carvacrol [1] (75.0%) linalool [5] (9.0%) thymol [4] (1.0%)	*Amblyomma americanum* (Acari: Ixodidae)	EO at 0.413 mg/cm^2 repelled 100% of the adults. EO at 0.103 mg/cm^2 repelled 67% of the adults. 0.075 mg/cm^2 thymol repelled 67% of the adults. 0.075 mg/cm^2 carvacrol repelled 29% of the adults.		Carroll et al. (2017)
[d]*Origanum vulgare*	carvacrol [1] *p*-cymene [3] thymol [4]	*Psoroptes cuniculi* (Acari: Psoroptidae)	0.05 and 0.02% of the EO caused total mortality at 1 and 6h. 1 mg/mL of carvacrol caused mortality in 84.0% of the adults. 0.2 mg/mL thymol caused mortality in 96.0% of the adults. 1% *p*-cymene caused mortality in 66.0% of the adults.	X	Shang et al. (2016)
	carvacrol [1] (75.0%)	*Haemaphysalis longicornis* (Acari: Ixodidae)	LC_{50} = 43.50 mg/mL and LC_{95} = 113.66 mg/mL for adults.		Qiao et al. (2021)
Origanum floribudum	carvacrol [1] (46.0%)	*Hyalomma scupense* (Acari: Ixodidae)	EO LC_{50} = 0.131 µL/mL as a larvicide.	X	
Rosmarinus officinalis	camphor [6] (35.0%) 1,8-cineole [8] (24.0%) borneol [9] (22.5%)	*Tetranychus urticae* (Acari: Tetranychidae)	EO at 20 mL/L is 63% ovicidal. EO at 10 mL/L showed 98% mortality in adults at 96h. EO at 20 mL/L caused mortality in 94% of the nymphs at 96h.	X	Salman et al. (2015)
	α-pinene [7] (31.0%) verbenone [10] (15.0%) 1,8-cineole [8] (14.0%)	*R. microplus*	EO at 85% was larvicidal.		Martinez-Velazquez et al. (2011)
	1,8-cineole [8] (26.0%), α-pinene [7] (18.0%), camphor [6] (17.0%) camphene [11] (11.0%) *β*-myrcene [12] (9.0%) bornyl acetate [13] (4.0%)	*T. urticae*	EO at 0.25% caused mortality in 100% of the eggs at 1h.		Laborda et al. (2013)
	borneol [9] (21.0% α-pinene [7] (15.0%) α-terpineol [14] (7.0%) camphene [11] (7.0%) *p*-cymene [3] (6.0%) *β*-pinene [15] (5.0%)	*Tetranychus turkestani* (Acari: Tetranychidae)	LC_{50} of the EO = 30% µL/L for adults. LC_{95} of the EO = 36% µL/L for adults.		Zandi-Sohani and Ramezani (2015)
	Not reported	*T. urticae*	EO caused 61% mortality in larvae. EO inflicted 53% mortality in adults.		Attia et al. (2012)
	1,8 cineole [8] (31,5%) Canphor [6] (20,0%) α-pinene [7] (17.5%)	*T. urticae*	LC_{50} of the EO = 10.0 ml/L for adults in bean plants LC_{50} of the EO = 13.0 ml/L for adults in tomato plants		Miresmailli et al. (2006)
Salvia officinalis	1,8-cineole [8] (26.0%) α-thujone [16] (42.0%) camphor [6] (11.0%) 1,8-cineole [8] (10.0%) α-pinene [7] (6.0%) *β*-thujone [17] (6.0%) camphene [11] (6.0%) *β*-pinene [15] (3.0%) *β* -myrcene [12] (1.4%)	*H. scupense* *T. urticae*	EO LC_{50} = 0.108 µL/mL against larvae. EO at 0.25% caused 100% mortality in adults.		Djebir et al. (2019) Laborda et al. (2013)
	1,8-cineole [8] (34.0%) camphor [6] (29.0%) α-thujone [16] (19.0%)	*T. urticae*	EO at 20 mL/L caused mortality in 50% of the eggs. 10 mL/L caused mortality in 97% of		Salman et al. (2015)

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4

Table 1 (*continued*)

Lamiaceae species	Major compounds[a]	Mite/tick species	Biological activities	Plants EO containing candidate insecticides[b]	Reference(s)
			adults at 96h. 20 mL/L caused mortality in 91% of the nymphs at 96h. 10 mL/L repelled 45% of the adults at 24h.		
	Not reported	*T. urticae*	EO caused mortality in 61% of the larvae. EO caused mortality in 57% of the adults.		Attia et al. (2012)
	Not reported	*V. destructor*	EO at 5% caused mortality in 6% of adults.		Bendifallah et al. (2018)
Mentha pulegium	piperitone [19] (32.0%) piperitenone [20] (29.0%) α-terpineol [14] (6.0%) borneol [9] (4.0%)	*T. urticae*	EO LC$_{50}$ = 14.5 μL/L, LC$_{95}$ = 19.9 μL/L for adults. LC$_{50}$ = 2.57 μL/L against eggs. LC$_{50}$ = 2.25 μL/L against adults.		(Zandi-Sohani and Ramezani, 2015; Mozaffari et al., 2013)
	pulegone [25] (41.0%) menthone [30] (28.0%)	*T. urticae*	EO caused mortality in 90% of larvae and 91% of adults.		Attia et al. (2012)
Mentha piperita	Not reported	*T. urticae*	EO LC$_{50}$ = 15.86 mg/L against adults. 100% adults repelled with LC$_{50}$ = 455.41 ppm.		(El-Zemity et al., 2009; Reddy and Dolma, 2018)
Mentha viridis	carvone [18] (51.0%) limonene [21] (21.0%)	*T. turkestani*	EO LC$_{50}$ = 15.3 μL/L against adults. LC$_{95}$ = 23.4 μL/L against adults.		Zandi-Sohani and Ramezani (2015)
Mentha longifolia	Not reported	*T.turkestani*	EO LC$_{50}$ = 11.08 mg/L against adults. 100% of adults were repelled.		Reddy and Dolma (2018)
Cunila microcephala	menthofuran [22] (72.0%) 1,8-cineole [8] (9.0%)	*R. microplus*	EO at 10 μL/mL caused mortality in 5% of larvae.		Apel et al. (2009)
Cunila incisa	1,8-cineole [8] (55.0%) sabinene [23] (9.0%) borneol [9] (6.0%) α-terpineol [14] (5.0%) α-pinene [7] (3.0%) terpinen-4-ol [24] (3.0%)	*R.microplus*	EO at 10 μL/mL caused mortality in 18% of larvae.		Apel et al. (2009)
Cunila spicata	menthofuran [22] (34.0%) borneol [9] (19.0%) limonene [21] (12.0%) camphene [11] (9.0%) α-pinene [7] (4.0%)	*R.microplus*	EO at 10 μL/mL caused mortality in 100% of larvae.		Apel et al. (2009)
Cunila angustifólia	sabinene [23] (32.0%) o-cymene [28] (8.5%) limonene [21] (7.0%)	*R. microplus*	EO at 10 μL/mL caused 99% mortality to larvae.		Apel et al. (2009)
Cunila incana	β-pinene [15] (27.0%) α-pinene [7] (26.0%) limonene [21] (7.0%)	*R. microplus*	EO at 10 μL/mL caused mortality in 99% of larvae.		Apel et al. (2009)
Hesperozygis ringes	menthofuran [22] (34.0%) borneol [9] (19.0%)	*R.microplus*	EO LC$_{50}$ = 0.260 μL/mL, LC$_{90}$ = 0.541 μL/mL against adults. Pulegone LC$_{50}$ = 0.321 μL/mL, LC$_{90}$ = 0.602 μL/mL against adults.		Ribeiro et al. (2010)
Tetradenia riparia	pulegone [25] (86.0%)	*R.microplus*	EO LC$_{50}$ = 0.534 g/mL, LC$_{99}$ = 1.552 g/mL against adults. LC$_{50}$ = 1.222 g/mL, LC$_{99}$ = 11.382 g/mL against larvae.		Gazim et al. (2011)
Vitex gardneriana	terpinen-4-ol [24] (0.2%) myrtenal [32] (0.2%) pinocarvone [29] (0.1%) α-terpineol [14] (0.1%)	*Aceria guerreronis* (Acari: Eriophyidae)	EO LC$_{50}$ = 0.85 mg/mL against adults.		De Sena Filho et al. (2017)
Vitex rufescens	limonene [21] (0.1%)	*A. guerreronis*	None reported		De Sena Filho et al. (2017)
Vitex capitata	limonene [21] (0.1%) linalool [5] (0.2%) α-terpineol [14] (0.1%)	*A. guerreronis*	None reported	X	De Sena Filho et al. (2017)
Vitex megapotamica	linalool [5] (1.1%), α-terpineol [14] (0.2%)	*A. guerreronis*	None reported	X	De Sena Filho et al. (2017)
Lavandula angustifolia	linalool [5] (42.0%) isoborneol [26] (9.0%) 1,8-cineole [8] (5.0%) camphor [6] (4.0%)	*T. urticae*	EO at 20 mL/L caused mortality in 79% of the eggs. 20 mL/L caused mortality in 92% of the adults at 96h. 10 mL/L repelled 93% of the adults at 48h. 10 mL/L repelled 100% of the nymphs at 6h.	X	Salman et al. (2015)
	Not reported	*H. marginatum rufipes*	Repelled 70–100% of the adults.		Mkolo and Magano (2007)
	Not reported	*R. annulatus*	EO at 6% and 8% caused mortality in 100% of adult females and 100% of the eggs.		Pirali-Kheirabadi and Teixeira da Silva (2010)

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Table 1 (*continued*)

Lamiaceae species	Major compounds[a]	Mite/tick species	Biological activities	Plants EO containing candidate insecticides[b]	Reference(s)
Lavandula stoechas	α-thujone [16] (65.0%) camphor [6] (18.0%) 1,8-cineole [8] (7.0%)	*T. urticae*	EO at 15 µg/mL caused 100% mortality in adults. LC$_{50}$ = 2.92 µg/mL, LC$_{90}$ = 13.0 µg/mL against adults.		Sertkaya et al. (2010)
	α-thujone [16] (25.0%)	*H. scupense*	EO LC$_{50}$ = 0.253 µL/mL against larvae.		Djebir et al. (2019)
Ocimum suave	Not reported	*Rhipicephalus appendiculatus* (Acari: Ixodidae)	EO LC$_{50}$ = 0.024% against larvae. EO at 5% caused mortality in 94% of nymphs. EO at 10% caused mortality in 77% of adults.		Mwangi et al. (1995)
Ocimum basilicum	Not reported	*T. urticae*	EO at 3.20 µL/L caused 40% mortality against adults at 24h, 60% mortality against adults at 48h, 60% mortality against adults at 96h.		Jiang et al. (2018)
	Not reported	*T. urticae*	EO LC$_{50}$ = 5% of adults. EO LC$_{90}$ = 12% of adults at 72 h.		Keskin et al. (2020)
**Thymus satureioides*	borneol [9] (20.0%–48.0%) α-terpineol [14] (5.0%–18.0%) camphene [11] (5.0%–14.0%) α-pinene [7] (2.%-7.0%) thymol [4] (0.14%–8.0%) carvacrol [1] (0.88%–15.0%)	*T. cinnabarinus*	EO caused mortality in 50–94% of adults.	X	Sertkaya et al. (2010)
Thymus capitatus	carvacrol [1] (78.0%)	*H. scupense*	EO LC$_{50}$ = 0.058 µL/mL against larvae.	X	Djebir et al. (2019)
Thymus mongolicus	thymol [4] (34.0%)	*Haemaphysalis longicornis Neuman (Acari:* Ixodidae)			Qiao et al. (2021)
Hyptis verticillata	Not reported	*R. microplus*	Active against eggs and adults.		Facey et al. (2005)
Hyptis crenata	borneol [9] (17.0%) 1,8-cineole [8] (15.0%) *p*-cymene [3] (7.0%)	*R. microplus*	EO at 2.5% caused mortality in 94% of the eggs.		Povoa Violante et al. (2012)
Hyptis suaveolens	Not reported	*Amblyomma cajannense* (Acari: Ixodidae)	EO LC$_{50}$ = 0.550 mg/mL against adults. LC$_{90}$ = 2.117 mg/cm^2 against adults.		Soares et al. (2010)
Thymbra spicata L	carvacrol [1] (70.0%) *p*-cymene [3] (13.0%) γ-terpinene [2] (6.0%)	*T. cinnabarinus*	EO LC$_{50}$ = 0.53 µg/mL against adults. LC90 = 1.83 µg/mL against adults.	X	Sertkaya et al. (2010)
Thymbra capitata	Not reported	*T. urticae*	EO at 35% caused mortality in 61% of the larvae, mortality in 52.0% of the adults.		Attia et al. (2012)
Satureja hortensis L	carvacrol [1] (38.0%) γ-terpinene [2] (22.0%) *p*-cymene [3] (9.0%)	*T. turkestani*	EO LC$_{50}$ = 9.4 µL/L against adults.	X	Zandi-Sohani and Ramezani (2015)
	Not reported	*T. urticae*	EO LC$_{50}$ = 9.95 µL/L against adults at 24h. EO encapsulated in chitosan/tripolyphosphate nanoparticles LC$_{50}$ = 46.98 µL/L against adults at 24h. EO LC$_{50}$ = 2.02 µL/L against adults at 72h. EO encapsulated in chitosan/tripolyphosphate nanoparticles LC$_{50}$ = 31.30 µL/L against adults at 72h. EO LC$_{50}$ = 6.71 µL/L for eggs at 24h. EO encapsulated in chitosan/tripolyphosphate nanoparticles LC$_{50}$ = 211.66 µL/L for eggs at 24h.		Ahmadi et al. (2018)
	thymol [4] 1,8-cineole [8]	*T. urticae*	EO LC$_{50}$ = 0.876 µL/L against adults (contact) LC$_{50}$ = 7.074 µL/L against adults (fumigation).		Ebadollahi et al. (2015)
Zataria multiflora	thymol [4] (30.0%) carvacrol [1] (22.0%) *p*-cymene [3] (7.0%) γ-terpinene [2] (6.0%) linalool [5] (5.0%)	*T.turkestani*	EO LC$_{50}$ = 5.5 µL/L against adults. LC9$_{95}$ = 11.8 µL/L against adults.	X	Zandi-Sohani and Ramezani (2015)
Teucrium polium	Not reported	*T. urticae*	EO LC$_{50}$ = 1.784 µL/L against adults (contact). LC$_{50}$ = 5.395 µL/L adults (fumigation).		Ebadollahi et al. (2015)
Hyssopus officinalis	β-phellandrene [27] (78.0%) β-myrcene [12] (19.0%) β-pinene [7] (2.0%)	*T. urticae*	EO at 20 mL/L caused 51% mortality against eggs at 96h. EO at 20 mL/L caused mortality in 97% of the adults at 96h.		Salman et al. (2015)

Table 1 (*continued*)

Lamiaceae species	Major compounds[a]	Mite/tick species	Biological activities	Plants EO containing candidate insecticides[b]	Reference(s)
Micromeria fruticosa	Not reported	*T. urticae*	EO at 20 mL/L caused mortality in 98% of the nymphs at 96h. EO at 2.0 μL/L caused mortality in 63% of the adults at 24h. EO at 2.0 μL/L caused mortality in 83% of the adults at 48h.		Çalmaşur et al. (2006)
Nepta racemosa	Not reported	*T. urticae*	EO at 2.0 μL/L caused mortality in 60% of the adults at 24h. EO at 2.0 μL/L caused mortality in 85% of the adults at 48h.		Çalmaşur et al. (2006)
Hesperozygis ringens	pulegone [25] (86.0%)	*R. microplus*	EO LC_{50} = 0.260 μL/mL against adults. EO $LC_{99.9}$ = 0.541 μL/mL against adults.		Ribeiro et al. (2010)
Dorystoechas hastate	Not reported	*R. turanicus*	EO at 1% caused 100% mortality in larvae.		Koc et al. (2012)

[a] Number in brackets after compound refers to compound structure in Fig. 1.
[b] EO = Essential Oil; candidate compounds include carvacrol, linalool and bornyl acetate.
[c] Metabolites vary according to the phenological season.
[d] Tested with purchased majorities.

Tetranychus turkestani (Ugarov & Nikolskii) (Acari: Tetranychidae) as well as the ticks *R. microplus* and *H. scupense* (Table 1) (Attia et al., 2012; Martinez-Velazquez et al., 2011; Laborda et al., 2013; Zandi-Sohani and Ramezani, 2015; Yorulmaz Salman et al., 2014).

EOs extracted from the aerial parts of *Salvia officinalis* L. contain α-thujone (Compound 16, Fig. 1) as one of the major components, with high variation shown between studies (20.0–42.0%) (Laborda et al., 2013; Salman et al., 2015). Another study (Salman et al., 2015) identified a higher amount of 1,8-cineole (34.0%), while others have reported amounts varying from 10.0 to 34.0% (Martinez-Velazquez et al., 2011; Laborda et al., 2013; Yorulmaz Salman et al., 2014; Salman et al., 2015). In addition, *S. officinalis* biosynthesizes smaller amounts of the monoterpenes α-pinene, β-thujone (Compound 17, Fig. 1), camphene, and β-pinene (Supplementary Table 1) (Laborda et al., 2013). *S. officinalis* EOs were active against the mites *T. urticae* and *V. destructor* (Table 1) (Attia et al., 2012; Laborda et al., 2013; Zandi-Sohani and Ramezani, 2015; Salman et al., 2015; Bendifallah et al., 2018).

2.3. Mentha

Four species from the genus *Mentha* have been characterized for their chemical components in extracted EOs; all species were biologically active against *Tetranychidae* mites (Table 1). Piperitone (Compound 19, Figs. 1, 32.0%) and piperitenone (Compound 20, Figs. 1, 29.0%), along with smaller amounts of α-terpineol (6.40%) and borneol (4.77%), were present in the EO from aerial parts of *Mentha pulegium* L. (Zandi-Sohani and Ramezani, 2015) A median lethal concentration (LC_{50}) of 2.57 μL. L^{-1} of *M. pulegium* EO inflicted 80.0% mortality over 24 h in adults and impaired larval development of *T. urticae* (Table 1) (Zandi-Sohani and Ramezani, 2015; Mozaffari et al., 2013).

Mentha piperita L. was also active against *T. urticae* as measured in toxicity and repellency bioassays (El-Zemity et al., 2009; Reddy and Dolma, 2018). Carvone (Compound 18, Figs. 1, 51.0%) and limonene (Compound 21, Figs. 1, 21.0%) were the main compounds in EOs from aerial parts of *M. viridis*, which exhibited acaricidal action against *T. urticae* (Zandi-Sohani and Ramezani, 2015). The EOs extracted from the aerial leaves of *Mentha longifolia* L. were also active against *T. urticae* (Table 1) (Reddy and Dolma, 2018). Similarly, *M. viridis* and *M. longifolia* were toxic against the mite *T. turkestani* (Zandi-Sohani and Ramezani, 2015).

2.4. Cunila, Hesperozygis, and Tetradenia

A total of five species from the genus *Cunila* have been described as

toxic to the tick *R. microplus* (Apel et al., 2009). *Cunila microcephala* (Benth.), which is mainly composed of menthofuran (Compound 22, Figs. 1, 72.0%), along with smaller amounts of 1,8-cineole, exhibited minimal acaricidal activity against the tick *R. micropilus* (Apel et al., 2009). In addition, *Cunila incisa* (Benth.) contains 1,8-cineole (54.0%) as its primary metabolite, with sabinene (Compound 23, Fig. 1), borneol, α-terpineol, α-pinene, and terpinen-4-ol (Compound 24, Fig. 1) as minor components. Terpinen-4-ol (3%) was only found in *C. incisa* (Apel et al., 2009). EO from *C. incisa* also exhibited minimal acaricidal activity against *R. micropilus*, yet was relatively successful in exterminating this pest in comparison to *C. microcephala* (18% versus 5% mortality with application of 10 μL/mL) (Table 1) (Apel et al., 2009).

Cunila spicata (Benth.) produced menthofuran (34.0%) and borneol (19.0%) in its EO extract which were highly effective against *R. microplus* (10 μL/mL killed 100% of the larvae). In addition to menthofuran and borneol, lower concentrations of limonene (12.0%), camphene (9%), and α-pinene (4.0%) were detected (Apel et al., 2009). *Cunila angustifolia* (Benth.) contained sabinene (32.0%) as the main component and limonene (7.0%) as one of the minor compounds. Its EO was also active against *R. microplus*, causing 99% mortality in larvae at a concentration of 10 μL/mL. In addition, *Cunila incana* Benth. contained β-pinene and α-pinene as its primary components (27.0% and 26.0%, respectively), along with limonene (7.0%). This species exhibited similar acaricidal activity against *R. micropilus* as *C. spicata* and *C. angustifolia* species (Table 1) (Apel et al., 2009).

EO extracted from the leaves of *Hesperozygis ringes* (Benth Epling) consisted mainly of menthofuran (34%). *H. ringes* EO and pulegone (Compound 25, Fig. 1) were active against larvae and adults of *R. microplus* (Table 1) (Ribeiro et al., 2010; Facey et al., 2005). On the other hand, pulegone was identified as the major compound of *Tetradenia riparia* (Hochst Codd) EO (86.0%), which exhibited lower acaricidal activity than *H. ringes* in addition to a reduction in oviposition and their hatchability (Gazim et al., 2011).

2.5. Vitex

Vitex gardneriana (Schauer) volatiles were found to be toxic to the coconut mite *A. guerreronis* (LC_{50} = 0.85 mg/mL) (De Sena Filho et al., 2017). However, it was not possible to estimate the LC_{50} for *Vitex rufescens* (A.Juss), *Vitex capitata* (Vahl), or *Vitex megapotamica* (Spreng. Moldenke), as concentrations as high as 2.3 mg mL^{-1} were only mildly toxic to this mite. The toxicity of *V. gardneriana* against *A. guerreronis* is possibly caused by the presence of the sesquiterpenes 6,9-guaiadiene (19.0%), *L*-calamenene (13.0%), and caryophyllene oxide (18.0%),

which were the major components found in the EO of this plant (Table 1) (De Sena Filho et al., 2017).

2.6. Lavandula, Ocimum, and Thymus

The EO from the aerial parts of *Lavandula angustifolia* (Mill) were active against the mite *T. urticae* and the ticks *Hyalomma marginatum rufipes* (Koch), *Rhipicephalus (Boophilus) annulatus* (Say), and *H. scupense* (Salman et al., 2015; Mkolo and Magano, 2007; Pirali-Kheirabadi and Teixeira da Silva, 2010). Linalool (42.0%) was the major component, followed by isoborneol (9.0%), 1,8-cineole (5.0%) and camphor (4.4%). The EO from leaves of *Lavandula stoechas* L. consisted mainly of α-thujone (65.0%) and was active against *T. urticae;* smaller proportions of camphor and 1,8-cineole were detected as well (Djebir et al., 2019; Sertkaya et al., 2010). *L. stoechas* exhibited potent acaricidal activity against *H. scupense*, most likely attributed to its high concentration of α-thujone (Table 1) (Silva et al., 2004; Salman et al., 2015; Mkolo and Magano, 2007; Pirali-Kheirabadi and Teixeira da Silva, 2010).

Although their chemical compositions were not reported, the EO extracted from leaves of *Ocimum suave* and *Ocimum basilicum* (Linn.) were active against the tick *Rhipicephalus appendiculatus* (Neumann) (Acari: Ixodidae) and the mite *T. urticae* (Case et al., 2005; Mwangi et al., 1995; Keskin et al., 2020) .

The EO from the aerial parts of *Thymus satureioides* (Coss. & Ball.) consisted mainly of borneol (20.0–48.0%), with α-terpineol (18.0%), camphene (14.0%), α-pinene (7.0%), thymol (8.0%), and carvacrol (15.0%) making up more minor proportions. This species was active against the carmine spider mite *T. cinnabarinus* (Ramzi et al., 2017). *Thymus mongolicus* (Ronn) EO showed thymol (34.0%) as its main component and was toxic to *Haemaphysalis longicornis* Neumann (Acari: Ixodidae) 64.

2.7. Hyptis and Thymbra

A few species from the *Hyptis* and *Thymbra* genera have proven to be toxic against ticks of the family Ixodidae, particularly those belonging to the genera *Rhipicephalus*, and to *Tetranychus* mites. *Hyptis verticillata* (Jacquin) and *Hyptis crenata* (Pohl ex Benth.) were shown to be active against the tick *R. microplus*[72]. Similarly, the EO from aerial parts of *Hyptis suaveolens* (L. Poit) repelled the tick *Amblyomma cajannense* Fabricius [80]. Borneol (17.0%) and 1,8-cineole (15.0%) were the major components in the EOs extracted from the aerial parts of *H. crenata*, followed by smaller amounts of *p*-cymene (Table 1) (Attia et al., 2012; Sertkaya et al., 2010; Povoa Violante et al., 2012).

Regarding the genus *Thymbra*, two studies with biologically active species against mites of the family Tetranychidae were found. Carvacrol (70.0%) was the main component from the aerial parts of *Thymbra spicata* L., which was active against *T. cinnabarinus*. In addition to carvacrol, this species produced smaller amounts of *p*-cymene and γ-terpinene. *Thymbra capitata* EO showed activity against *T. urticae* (Table 1) (Sertkaya et al., 2010).

2.8. Satureja, Teucrium, Zataria, Hyssopus, and Nepta

For the genus *Satureja*, three studies are summarized here. The EO extracted from aerial parts of *Satureja hortensis* L. consisted of carvacrol (38.0%) and γ-terpinene (22.0%) as major components. The EO was active against the spider mites *T. turkestani* and *T. urticae* (Table 1) (Zandi-Sohani and Ramezani, 2015; Ahmadi et al., 2018; Ebadollahi et al., 2015).

The EO from aerial portions of *Zataria multiflora* (Boiss. ZM) showed thymol (30.0%) and carvacrol (22.0%) to be the major constituents, followed by minor amounts of *p*-cymene, γ-terpinene, and linalool. *Z. multiflora* along with *Teucrium polium* L. EOs were active against two spider mites (*T. turkestani* and *T. urticae*) (Table 1) (Zandi-Sohani and Ramezani, 2015; Ebadollahi et al., 2015).

The EO from the aerial parts of *Hyssopus officinalis* L. consisted mainly of β-phellandrene (78.0%) followed by β-myrcene (19.0%) and β-pinene (2.0%) as minor components (Salman et al., 2015). *H. officinalis* EO, as well as those of *Micromeria fruticosa* (L. Druce) and *Nepta racemose* (Lam.), were active against *T. urticae*. The EO extracted from the aerial parts of *Dorytoechas hastata* (Boiss et Heldr. ex Bentham) were highly active against *R. turanicus* (Table 1) (Çalmaşur et al., 2006; Salman et al., 2015; Ribeiro et al., 2010; Koc et al., 2012).

3. Mechanisms of action of volatile essential oils and their monoterpene components

The mechanism of action of many synthetic chemical pesticides, including organophosphates (OPs), abamectin and carbamates, involves inhibition of enzymatic processes. For example, blocking AChE cholinergic synapses of arthropod nervous systems reduces their movement and suppresses reproductive cycles (Silva et al., 2004; Nasr, 2015; Houghton et al., 2006). Alternatively, binding to the ionotropic γ-aminobutyric acid (GABA) primary receptor and inhibiting its binding to glutamate-dependent chloride channels (GluCls) blocks synaptic transmission in the arthropod's nervous system (Wang et al., 2019; Wei et al., 2018).

AChE plays an important role in the mechanism of action of some monoterpenes in producing their acaricidal effect. Monoterpenes are lipophilic compounds that can interfere with various biological functions in arthropods (El-Minshawy et al., 2018; Lee et al., 1997; Brattsten, 1983). Previous studies have reported that the quantitative structure activity relationship (QSAR) model showed good agreement between estimated and experimentally measured toxicity (LC_{50}) for tested monoterpenes from various chemical classes, including hydrocarbons (α-pinene), alcohols (1,8-cineole and 4-terpineol), aldehydes ((−)-citronellal) and ketones (limonene and pulegone) using contact and fumigation techniques against *T. urticae* and AchE inhibition (Abdelgaleil et al., 2019). Results showed that cuminaldehyde, (−)linalool, (−)-limonene and menthol demonstrated strong AchE inhibition which coincides with the hydrophobicity of these compounds, allowing them to penetrate the active site of the enzyme more easily than other monoterpenes (Abdelgaleil et al., 2019). Another study tested six monoterpenes for AChE docking against *T. urticae* and showed pulegone to be the most active, followed by citronellal, limonene, 4-terpineol, α-pinene, and 1,8 cineole (Abdelgaleil et al., 2019).

Salvia officinalis and *S. lavandulaefolia* Vahl. EOs were effective in inhibiting AChE (Perry et al., 2010; Savelev et al., 2003). Although camphor was the major component in the essential oil of *S. lavandulaefolia,* minor compounds 1,8-cineole and α-pinene were more potent when evaluated separately against AChE from bovine erythrocytes (Houghton et al., 2006; Perry et al., 2010). In addition, there appears to be a synergism with mixtures of 1,8-cineole and α-pinene, although this relationship disappeared at very low concentrations (Savelev et al., 2003). Bicyclic monoterpenes with allylic methyl groups, such as (+)-*R*-pinene and (−)-*R*-pinene, exhibited strong AChE inhibition, while oxygenated compounds, such as bicyclic alcohols and ketones, were less efficient (Miyazawa and Yamafuji, 2005).

4. Monoterpenoids from Lamiaceae docked to *Drosophila melanogaster* AChE

The AChE receptor used here is expressed in the insect *Drosophila melanogaster* (Harel et al., 2000) and is considered a relevant drug target for a broad range of chemicals, both natural and man-made, due to its critical role in synaptic neurotransmission (Fig. 2) (Millard and Broomfield, 1995). The active site of insect AChEs comprises three domains. The catalytic triad site hydrolyses acetylcholine to acetate and choline with involvement from the acyl pocket; the anionic site accommodates the positive quaternary amine of acetylcholine and other cationic substrates and inhibitors; and the oxyanion hole-forming
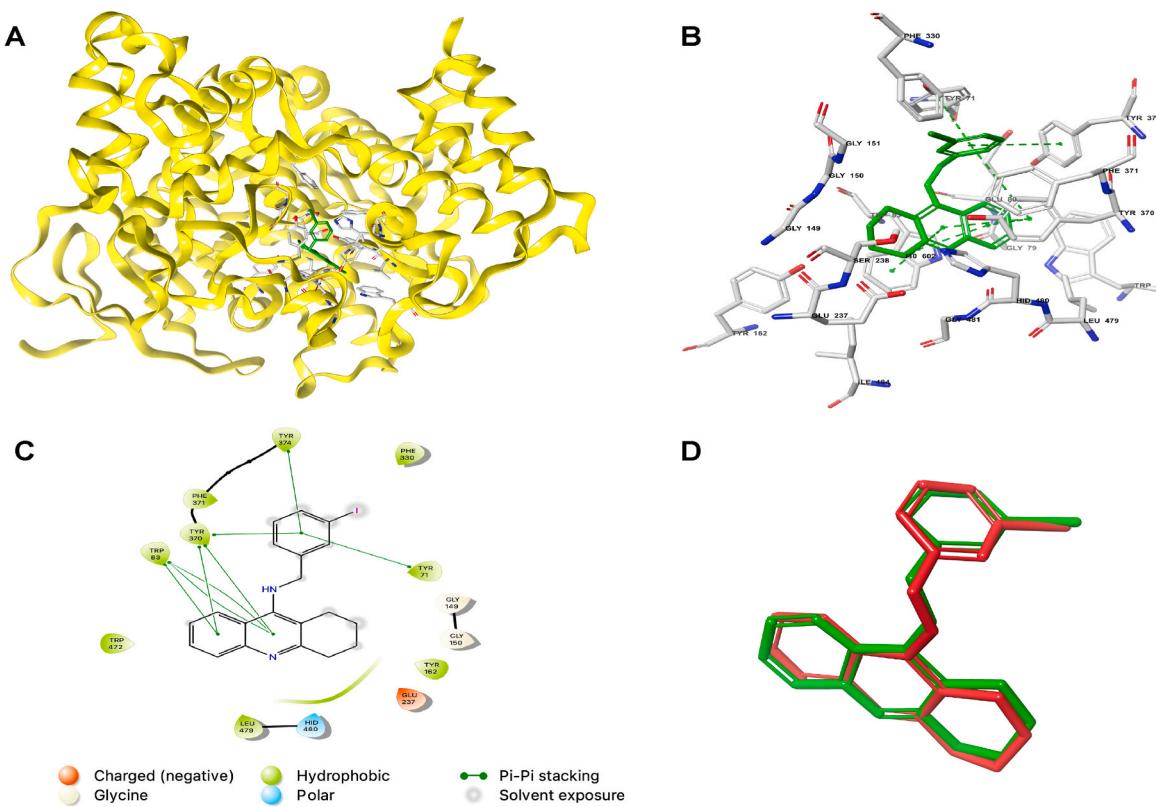
Fig. 2. Overview of the three-dimensional structure of the insect acetylcholinesterase (AChE) receptor (A) (PDB code: 1QON). The co-crystalized ligand tacrine (green) is shown in the binding pocket interacting with residues of the active site (B and C) and overlapped with the pose reproduction predicted by UCSF Dock 6. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

residues confer stability to the substrate-enzyme complex (Harel et al., 2000; Ordentlich et al., 1993). Here, we performed a structure-based virtual screening of 27 monoterpenes to identify compounds with the potential to produce binding profiles and orientations similar to those observed in a known insect AChE inhibitor, the tacrine derivative 9-(3-iodobenzylamino)-1,2,3,4-tetrahydroacridine (Harel et al., 2000) (see Supplementary Material for more information about the molecular docking procedures).

The ligand pose (binding mode) predicted by the docking model did not deviate spatially from the surface defined by the reference binder tacrine derivative (Fig. 2). The molecular footprint of the crystal ligand (reference) and the energy minimized ligand docked in the active site of AChE showed a substantial overlap on per-residue electrostatics and, to a lesser extent, Van der Waals footprints (Supplementary Material). The parameter used to validate the docking model was the RMSD (root mean square deviation) of the cartesian coordinates of the ligand atoms in the docked and crystallographic conformations (Morris and Lim-Wilby, 2008).

Superimposing the docked ligand onto the crystal structure of the tacrine derivative resulted in a RMSD of 0.43 Å (RMSD <2 Å indicates a model of correct topology), confirming that the pose reproduction agreed with the crystal structure of the reference binder. The binding energy (grid score, Kcal/mol) of the ligand-receptor complex were −68.26 kcal/mol (rigid docking), −66.79 kcal/mol (fixed anchor docking), and −63.23 kcal/mol (flexible docking).

The most probable binding sites of the monoterpenes to the insect AChE receptor are in the vicinities of Tyr71, Trp83, Trp472, Phe330, Tyr370, and His480 (Fig. 2B). The interactions of the monoterpenes with the residues mentioned above indicate that they appear to have a reversible binding mode to both the free enzyme and the enzyme-substrate complex (Svobodova et al., 2019). Reversible inhibitors usually interact with the allosteric peripheral anionic site, causing

conformational changes in the enzyme that decrease its affinity for acetylcholine (Bourne et al., 2016; Silman and Sussman, 2008; Ripoll et al., 1993; Tan et al., 1993). The docking results presented herein are in line with previous research, which showed that nine aromatic residues in the active-site gorge of AChE changed their conformations upon insertion of the reversible inhibitor tacrine, with a whole side chain of residues (including Tyr71, Trp83, Tyr324, Phe330, Tyr370, Phe371, Tyr374, Trp472, and His480) moving after ligand binding to permit accommodation of the inhibitor (Harel et al., 2000).

The docking simulations showed that linalool, carvacrol, and bornyl acetate exhibited the most favorable energy grid scores when compared to the other 24 compounds (approximately −40 kcal/mol, −38 kcal/mol, and −37 kcal/mol, respectively) (Supplemental Table 1). These three compounds have the greatest potential to bind to the active site of AChE when compared to the other monoterpenes listed in the customized library of ligands (Table 1; Fig. 1). As for the structural similarity of the monoterpenes with the tacrine derivative, we used similarity-based functions to rescore the compounds based on their spatial similarity to the 3D geometry of the reference binder (Allen and Rizzo, 2014). The Hungarian matching score considers the chemical composition and the 3D position and conformation of the candidate ligand and the reference binder, where a positive value close to zero – or a large negative value – indicate a favorable overlap between the two molecules (Allen and Rizzo, 2014). After the monoterpenes were rescored based on the similarity-based functions, the Hungarian matching score revealed that carvacrol overlapped more tightly with the crystallized ligand 9-(3-iodobenzylamino)-1,2,3,4-tetrahydroacridine than linalool and bornyl acetate (Supplemental Table 1).

We also searched for hydrogen-bonding interactions between the monoterpenes and the binding pocket of *D. melanogaster* AChE. Based on the docking simulation results, we predicted that linalool was the only compound to form hydrogen bonds with the active site cleft of AChE
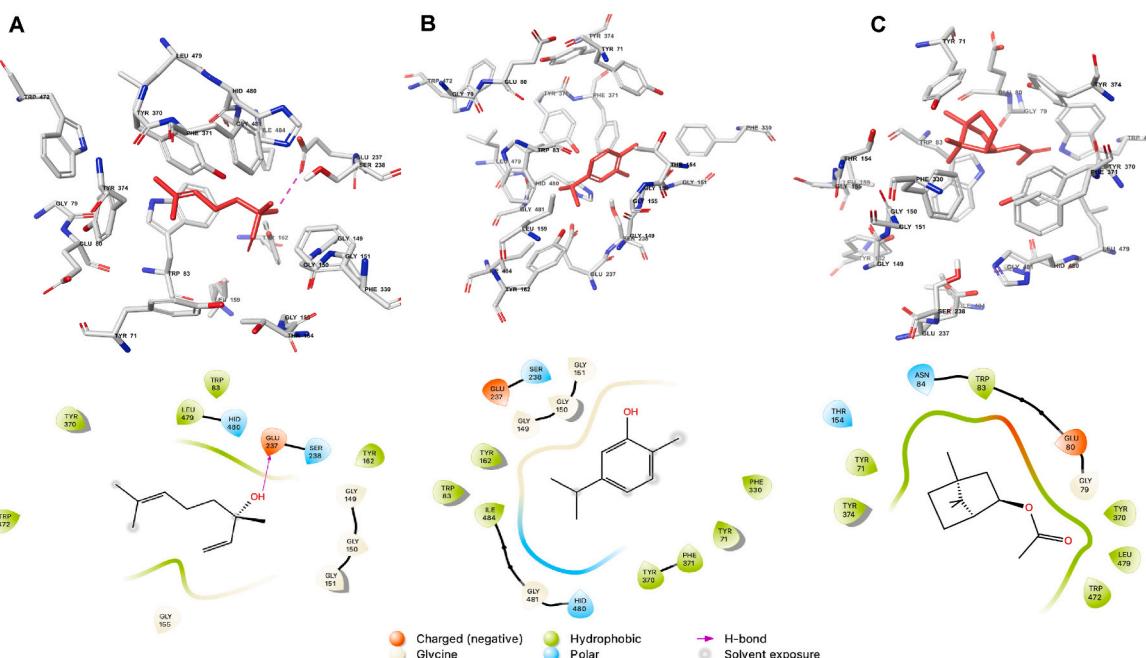
Fig. 3. Linalool (A), carvacrol (B), and bornyl acetate (C) docked in the binding pocket of *D. melanogaster* acetylcholinesterase (AChE). Linalool in the binding pocket shows bonding to the catalytic triad, oxy-anion hole residues with H bond in GLU237 (A). Carvacrol and bornyl acetate do not have a H-bond in the binding pocket of the receptor (B and C). Interactions within a reasonable H-bonding distance (2.0–3.5 Å) are shown as a purple line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 3). Linalool produced a hydrogen bond (defined as the distance between acceptor and donor of ~2.4 Å) with Glu237 (Fig. 3A). The catalytic triad residues (Glu237, Ser238, and His480) are involved in the stabilization and orientation of tacrine derivatives in the binding pocket of AChE (Svobodova et al., 2019). Disruption of the catalytic triad by a reversible inhibitor causes a failure in the acyl-binding pocket that affects the release of acetic acid and regeneration of the free enzyme (Nachon et al., 2020). Our *in-silico* simulation suggests that the binding mode of linalool, carvacrol and bornyl acetate to the active site of AChE is similar to that of the tacrine derivative and could be further explored in structure-activity relationship studies to test its specific affinity against insect cholinesterases.

Also, our docking simulation is intended to be stable using AchE from other species. Indeed, previous work showed the AchE catalytic triad (choline binding sites, an oxyanion hole and acyl pocket) presented in two aces genes (Prace1 and Prace2) encoded acetylcholinesterase identified from *Pieris rapae*, consisting of 694 and 638 aa respectively (Jiang et al., 2018) (Fig. 3 and Supplementary Material).

This review provides comprehensive, up-to-date information on the bioactivity of monoterpenes from Lamiaceae species against pest mites and ticks. Monoterpenes, specifically carvacrol, linalool, and bornyl acetate, were the most promising compounds identified from our literature review on acaricidal monoterpenes, when combined with the docking information. Targeted research in terpene biosynthesis could enhance the biotechnological production potential of bioactive monoterpenes, providing sustainable alternatives to the discovery of novel synthetic pesticides. Further, understanding the mechanism of toxicity to Acari may contribute to understanding the biochemistry of the aforementioned monoterpenes. New investigations should be carried out both with isolated monoterpenes and in combination to evaluate synergism or antagonism in order to find the best combination of monoterpenes for development effective acaricides.

Declaration of competing interest

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

Data availability

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2022.106126.

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