



## Nano- and microformulated botanicals for managing ticks and mites of medical and veterinary importance: Past, present, and future<sup>☆</sup>

Priscilla Farina<sup>a</sup>, Giulia Giunti<sup>b</sup>, Orlando Campolo<sup>c</sup>, Filippo Maggi<sup>d</sup>, Renato Ricciardi<sup>a</sup>, Andrea Lucchi<sup>a</sup>, Angelo Canale<sup>a</sup>, Roman Pavela<sup>e,f,g</sup>, Raul Narciso C. Guedes<sup>h</sup>, Nicolas Desneux<sup>i</sup>, Giovanni Benelli<sup>a,\*</sup>

<sup>a</sup> Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, Pisa 56124, Italy

<sup>b</sup> Department of Pharmacy, University of Salerno, Fisciano, Italy

<sup>c</sup> Department of Agriculture, University "Mediterranea" of Reggio Calabria, Reggio Calabria, Italy

<sup>d</sup> Chemistry Interdisciplinary Project (ChIP) research center, School of Pharmacy, University of Camerino, Camerino 62032, Italy

<sup>e</sup> Crop Research Institute, Drnovska 507, Prague 161 06, Czech Republic

<sup>f</sup> Department of Plant Protection, Czech University of Life Sciences Prague, Kamycka 129, Praha, Suchbát 165 00, Czech Republic

<sup>g</sup> Department of Plant Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea

<sup>h</sup> Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil

<sup>i</sup> Université Côte d'Azur, INRAE, CNRS, UMR ISA, Nice 06000, France

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### ABSTRACT

Developing effective and sustainable green solutions for managing arthropod pests and vectors is a timely challenge. The present review is dedicated to mites and ticks (Acari), species which represent a burden for human and animal health. The focus is on hard ticks (Ixodidae), primarily belonging to the genera *Haemaphysalis* spp., *Hyalomma* spp., and *Rhipicephalus* spp., house dust mites *Dermatophagoides* spp., stored-product mites *Tyrophagus putrescentiae* and *Acarus siro*, various strains of the itch mite *Sarcoptes scabiei*, the poultry red mite *Dermanyssus gallinae*, and the main ectoparasitic mite of honeybees *Varroa destructor*. We overviewed all the nano- and microemulsions and capsules loaded with essential oils, plant extracts, and/or singular compounds applied as toxicant or repellent treatments to manage such pests. Additionally, we explored current knowledge about the effectiveness of metal nanoparticles obtained through green synthesis routes by exploiting phytochemicals in botanical extracts. For all the products, we reported their mean particle size, tested formulation, efficacy, and when available, mode of action, comparison with synthetic acaricides, and non-target effects. Future challenges involve the need to improve the synthesis technologies and storage conditions of the different formulations; scaling up the production is necessary too to achieve lower prices and wider distribution. Notably, research on *S. scabiei* and *V. destructor* is required, as these species are extremely overlooked when compared to hard ticks and other mites.

### 1. Introduction

Mites and ticks are classically grouped under a taxon called Acari. Many of them pose significant burden for human and animal health, causing direct harm through feeding on their hosts (Magnarelli, 2009; Hwang, 2023), and indirectly by transmitting medical and veterinary important pathogens (Magnarelli, 2009; Valiente Moro et al., 2009; Niedringhaus et al., 2019; Benelli, 2020; Lester et al., 2022) or triggering allergic reactions (Arlan, 2002; Ahn et al., 2006; Sánchez-Borges et al.,

2013). As such, managing these pests is imperative for safety and well-being and should involve a holistic approach integrating biological, chemical, mechanical, and physical means (Benelli, 2016, 2019; Cafarchia et al., 2022).

Before the chemical synthesis boom in the 1940s, pest control was historically attempted using various organs from aromatic plants and their extracts or decoctions (Pavela, 2016). Synthetic acaricides have then played a pivotal role in pest management, but whose overuse has faced challenges such as resistance development, environmental

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\* Corresponding author.

E-mail address: [giovanni.benelli@unipi.it](mailto:giovanni.benelli@unipi.it) (G. Benelli).

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concerns, undesirable effects on non-target species, and possible presence of residues in meat, milk, and other products of animal origin (Agwunobi et al., 2021; Waldman et al., 2023). Still, the most common acaricides rely on neurotoxic compounds (George, 2000; Stará et al., 2011; Abbas et al., 2014), including organophosphates, organochlorines, pyrethroids, and some systemic parasiticides administered orally or topically to hosts (Sharaf, 2024). Repellents also offer a valuable option, particularly for personal protection (Benelli et al., 2019; Selles et al., 2021).

Emerging formulation technologies are coming as promising alternatives, leveraging eco-friendly botanical products, including essential oils (EOs), plant extracts, and their constituents (Pazinato et al., 2014; dos Santos et al., 2017; Benelli and Pavela, 2018; Choi et al., 2021; Ibrahim et al., 2022a; Kavallieratos et al., 2022 a,b). Furthermore, researchers are proposing new approaches to develop natural product-derived compounds with high efficacy (Choi et al., 2021; Anholetto et al., 2024). These botanical formulations, categorised as nano- or microformulations based on mean particle sizes of 1–100 nm or 1–1000 µm, respectively, demonstrate enhanced efficacy and safety profiles. Surfactants such as polysorbate 20 or 80, propylene glycol, sodium dodecyl sulphate, sodium dodecyl benzene sulfonate, or sorbitan monooleate are commonly employed in oil-in-water emulsions (dos Santos et al., 2017; Chaisri et al., 2019; Abdel-Ghany et al., 2021a). Some matrices used as encapsulants are chitosan, gelatin, polycaprolactone, poly(D, L-lactic-co-glycolic), polyethylene glycol, and β-cyclodextrin (Pereira Barbosa et al., 2016; Yim et al., 2016; dos Santos et al., 2017; Sabahi et al., 2020). All these formulations optimise the active ingredient (AI) delivery, stability, and solubility, minimising the need for the toxic organic solvents conventionally used (Athanasios et al., 2018).

The small particle size of nano- and microformulations facilitates efficient penetration through the arthropod cuticle, allowing AIs to reach their sites of action (Chaisri et al., 2019; Ibrahim et al., 2022a). Most of the EO components, known for their cytotoxic and neurotoxic effects in arthropods, exert their action through mechanisms such as acetylcholinesterase (AChE) inhibition and neurotransmitter modulations (e.g., γ-aminobutyric acid (GABA), octopamine, tyramine) (Jankowska et al., 2017; Ibrahim et al., 2022c). As for the repellence, Acari-host interaction is driven, among others, by volatile cues perceived by olfactory sensilla, but the precise molecular mechanism behind the repellent chemoreception is still poorly investigated (Bissinger and Roe, 2010). Understanding, for instance, the role of proteins and the central nervous system in olfaction would help designing targeted and innovative repellents, even of vegetal origin, able to “mask” the host odour and protect it.

Green, biological synthesis of nanoparticles (NPs) offers a cost-effective, biocompatible, and environmentally friendly approach, harnessing phytochemicals in plant extracts to reduce the metals or metal oxide precursors added and stabilise them (Gupta et al., 2023). The metal-salt and plant extract reaction mixture is then reduced, resulting in a colour change signalling the NP formation (Benelli et al., 2017; Ying et al., 2022). Metal NPs can either accelerate or decelerate some cellular mechanisms of host cells, like oxidative stress induction by creating reactive oxygen species (ROS) and decreasing antioxidant activity (Gattan et al., 2023; Majeed et al., 2023). Metal NPs can also penetrate cell walls compromising the permeability of cell membranes, causing denaturation of cytoplasmic ribosomal elements and altering the DNA structure (Elumalai et al., 2022; Alghamdi et al., 2024). Interestingly, metal NPs only exerted a minor negative impact on some non-target terrestrial and aquatic organisms (Zahir and Rahuman, 2012; Elumalai et al., 2022), but further investigations are mandatory.

This review focuses on medical and veterinary relevant Acari, including hard tick species associated with several hosts and mite species such as the house dust mites, stored-product mites, various strains of the itch mite *Sarcoptes scabiei*, the poultry red mite *Dermanyssus gallinae*, and the main ectoparasitic mite of honeybees *Varroa destructor*. We

overviewed the nano- and microformulations loaded with botanical-based AIs, their particle size, formulation, efficacy, and when available, mode of action, comparison with synthetic acaricides, and non-target effects. Furthermore, we explored other formulations of unknown size, but having a comparable composition and, in particular, showing promising potential for further improvement.

Future challenges involve the need to improve the synthesis technologies and storage conditions of the different formulations. Scaling up the production is necessary to achieve lower prices and wider distribution. Notably, research on *S. scabiei* and *V. destructor* is required, as these species are extremely overlooked when compared to hard ticks and other mites.

## 2. Toxicant and repellent botanical-based formulations against Acari

Essential oils, other plant extracts, some of their constituents, and metal NPs synthesised from green routes have garnered attention as potential pest control tools for over four decades (Pavela and Benelli, 2016). Ticks (Tables 1 and 2) and mites (Tables 3 and 4) of medical and veterinary relevance have been targeted by several studies involving botanical formulations. Overall, here are reported the results concerning the application of 25 different EOs and five plant extracts on the Acari species involved in our review (Tables 1, 3, and 4). Among these, *Syzygium aromaticum* (L.) Merr. & L.M. Perry EO and its main compound eugenol stand out as highly effective toxicants and repellents against medical and veterinary relevant Acari. *Thymus* spp. and its main compound thymol, tea tree oil from *Melaleuca alternifolia* (Maiden & Betche) Cheel, neem kernel oil from *Azadirachta indica* A.Juss., and *Origanum* spp., *Pelargonium* spp., and *Cinnamomum* spp. EOs also demonstrate significant efficacy.

Nanoemulsions are the most investigated, with 13 formulations tested against ticks and nine against mite species (Tables 1 and 4). Furthermore, five microemulsions (four on ticks and one on mites) and three nanocapsules (on ticks) were employed. Efficacy against house dust mites was also assessed for nine different microcapsules and two nanoparticles (Table 3). The metal NPs obtained from twenty plant extracts and evaluated on ticks (Table 2) predominantly feature silver (12 formulations), followed by zinc (five), copper (three), nickel (two), and titanium (two).

The mean dimensions and morphology of the nano- and microformulations are typically characterised using transmission electron microscopy or dynamic light scattering. Some papers also define particle size distribution through the polydispersity index, assess surface charge using zeta potential, evaluate colloidal stability and absorption spectrum, monitor potential chemical changes using Fourier transform infrared spectroscopy, and report viscosity, pH, and other parameters.

Formulations, particularly emulsions and metal NPs, are usually tested on the target pests via direct or indirect contact. In the former case, the specimens, mainly adults, are fully immersed (adult immersion test, AIT) in the specific treatment for from 30 s to 10 min. In the latter case, groups of pests, especially larvae, are placed on filter paper discs or inside filter paper packets (larval packet test, LPT) impregnated with the treatment for 24 or 48 h of incubation. LPT seems to possess high repeatability and is recommended by the Food and Agriculture Organization (FAO, 1971). Other modes of administration, such as ingestion (through treated blood), fumigation, or *in vivo* testing on host animals (e.g., cows, dogs, rabbits), are detailed in the above mentioned tables. Overall, on female ticks, effects on reproductive performance, including number of eggs laid, egg weight, and hatchability after the treatment, are frequently evaluated. A few works also investigate the repellence, intended as the ability to interrupt the host seeking behaviour of ticks, exerted by volatile botanical formulations under laboratory conditions. The repellent effect of the various botanical-loaded microcapsules on house dust mites is often assessed according to the American Association of Textile Chemists and Colorists (AATCC) test method 194–2007. The

**Table 1**

Effects of micro- and nanoformulations loaded with essential oils (EOs), plant extracts, single constituents, or their mixtures on hard ticks (Ixodida: Ixodidae).

Tick species	Botanical active ingredient	Family	Main compound	Formulation	Particle size	Effect	Notes	Reference
<i>Hy. dromedarii</i>	<i>Artemisia herba-alba</i> extract	Asteraceae		Nanoemulsion	62–69 nm	LC <sub>50</sub> by direct contact on eggs 0.3% LC <sub>50</sub> by direct contact on larvae 0.7% LC <sub>50</sub> by direct contact on engorged nymphs 0.3% LC <sub>50</sub> by direct contact on unfed adults 4.4% Negative effect on the reproductive performance	The oral administration of 1500 mg kg <sup>-1</sup> d <sup>-1</sup> for 5 d to healthy adult mice showed histopathological alterations in the liver and kidneys	Abdel-Ghany et al. (2021a)
<i>Hy. dromedarii</i>	<i>Melia azedarach</i> extract	Meliaceae		Nanoemulsion	52–91 nm	LC <sub>50</sub> by direct contact on eggs 1.1% LC <sub>50</sub> by direct contact on larvae 1.7% LC <sub>50</sub> by direct contact on engorged nymphs 0.4% LC <sub>50</sub> by direct contact on unfed adults 22.2% Negative effect on the reproductive performance	The oral administration of 1500 mg kg <sup>-1</sup> d <sup>-1</sup> for 5 d to healthy adult mice showed histopathological alterations in the liver and kidneys	Abdel-Ghany et al. (2021a)
<i>R. annulatus</i>	<i>Allium sativum</i> EO	Amaryllidaceae		Nanoemulsion	32.2–96.3 nm	At 5 mg L <sup>-1</sup> , 100% adult mortality after 24 h Morphological alterations to the mouthparts and dorsal and ventral cuticles	With phoxim, 100% adult mortality after 12 h	Abd-Elrahman et al. (2024)
<i>R. annulatus</i>	<i>Pelargonium graveolens</i> EO	Geraniaceae	Citronellol 14.44%	Nanoemulsion	17.84 nm	At 10.0%, 99.33 ± 1.00% mortality in eggs 100% mortality in larvae 96.66 ± 0.577% mortality in adult females Tick reduction by 87.97 ± 3.504% on infested cattle after 21 d	With deltamethrin, tick reduction by 29.88 ± 1.85% on infested cattle after 21 d	Ibrahim et al. (2022a)
<i>R. annulatus</i>	<i>Citrus × limon</i> EO	Rutaceae	(L)-alpha-Terpineol 18.32%	Nanoemulsion	50.66 nm	At 10.0%, 69.00 ± 3.605% egg mortality by indirect contact 8.666 ± 3.214% larval mortality by indirect contact 37.33 ± 3.055% adult mortality by direct contact	Phoxim at 0.50 µL mL <sup>-1</sup> was more effective on all the life stages	Ibrahim et al. (2022b)

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Table 1 (continued)

Tick species	Botanical active ingredient	Family	Main compound	Formulation	Particle size	Effect	Notes	Reference
<i>R. annulatus</i>	Tea tree ( <i>Melaleuca alternifolia</i> ) oil	Myrtaceae	Terpinene 52.24%	Nanoemulsion	188.4 nm	At 10.0%, 13.00 ± 2.00% egg mortality by indirect contact 100% larval mortality by indirect contact 76.33 ± 0.577% adult mortality by direct contact	Phoxim at 0.50 µL mL <sup>-1</sup> was more effective on all the life stages	Ibrahim et al. (2022b)
<i>R. annulatus</i>	d-Limonene			Nanoemulsion	258.5 nm	LC <sub>50</sub> by indirect contact on eggs 27.67% LC <sub>50</sub> by indirect contact on larvae 6.48% LC <sub>50</sub> by direct contact on adults 2.396%	AChE inhibition 27.33 ± 2.516% Phoxim at 0.50 µL mL <sup>-1</sup> was 100% effective on all the life stages but showed less AChE inhibition	Ibrahim et al. (2022c)
<i>R. australis</i>	Tea tree ( <i>Melaleuca alternifolia</i> ) oil	Myrtaceae	Terpinen-4-ol 40.8%	Microparticle	4.0 ± 0.03 µm	With 300 mg, 100% repellency on larvae for 3 d and about 45% after 15 d	Fast release formulation with weak cross-linking	Yim et al. (2016)
<i>R. australis</i>	Tea tree ( <i>Melaleuca alternifolia</i> ) oil	Myrtaceae	Terpinen-4-ol 40.8%	Microparticle	3.3 ± 0.02 µm	With 300 mg, max 38% repellency on larvae after 15 d	Slow release formulation with strong cross- linking	Yim et al. (2016)
<i>R. microplus</i>	6,7,8,9- Tetrahydrospilanthol			Nanoemulsion	145–175 nm	LC <sub>50</sub> by indirect contact on larvae 0.39 mg mL <sup>-1</sup> 100% efficacy in preventing egg laying and hatching from 2.50 mg mL <sup>-1</sup>		Anholetto et al. (2024)
<i>R. microplus</i>	Carvacrol			Microcapsule	4.5 ± 0.5 µm	LC <sub>50</sub> by direct contact on larvae 0.71 mg mL <sup>-1</sup> RC <sub>50</sub> on larvae 0.04 mg cm <sup>-2</sup> after 1 h and 0.05 mg cm <sup>-2</sup> after 3 h	RC <sub>50</sub> on larvae for DEET after 1, 3, and 6 h 0.006, 0.01, and 0.02 mg cm <sup>-2</sup>	da Silva Lima et al. (2017 and 2019)
<i>R. microplus</i>	<i>Cymbopogon winterianus</i> EO	Poaceae	Citronellal 31.4%	Microemulsion	19.6–47.3 nm	LC <sub>50</sub> by indirect contact on larvae 11.46%	w/w	Chaisri et al. (2019)
<i>R. microplus</i>	<i>Pilocarpus spicatus</i> EO	Rutaceae	Limonene 46.8%	Nanoemulsion	86.06 ± 0.2916 nm	At 50 mg mL <sup>-1</sup> , 97.14 ± 1.37% repellency on larvae after 6 h and 97.89 ± 0.52% after 10 h		Nogueira et al. (2020)
<i>R. microplus</i>	Tea tree ( <i>Melaleuca alternifolia</i> ) oil	Myrtaceae	Terpinen-4-ol 41.98%	Nanocapsule	287 ± 2 nm	100% reproductive inhibition from 0.375%	At 0.75%, not effective on infested cows 10% amitraz was less effective	Pazinato et al. (2014) Pazinatto Boito et al. (2016)
<i>R. microplus</i>	<i>Pouteria gardneriana</i> extract	Sapotaceae		Nanoparticle	589 nm	Significant egg mass reduction and female mortality by direct contact at 0.4% extract with 0.5 g chitosan nanoparticles		Pereira Barbosa et al. (2016)

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Table 1 (continued)

Tick species	Botanical active ingredient	Family	Main compound	Formulation	Particle size	Effect	Notes	Reference
<i>R. microplus</i>	<i>Cinnamomum verum</i> EO	Lauraceae	Cinnamaldehyde 41.27%	Nanoemulsion	175.6 nm	83.8% oviposition reduction at 5.0% Effective on infested cows after 20 d	10% cypermethrin was 54.2% effective	dos Santos et al. (2017)
<i>R. microplus</i>	<i>Cinnamomum verum</i> EO	Lauraceae	Cinnamaldehyde 41.27%	Nanocapsule	189.5 nm	73.6% oviposition reduction at 5.0% Effective on infested cows after 20 d	10% cypermethrin was 54.2% effective	dos Santos et al. (2017)
<i>R. microplus</i>	<i>Eucalyptus globulus</i> EO	Myrtaceae	1,8-Cineole 75.78%	Nanoemulsion	69 ± 0.71 nm	61.2% treatment efficacy at 5.0%		Galli et al. (2018)
<i>R. microplus</i>	<i>Eucalyptus globulus</i> EO	Myrtaceae	1,8-Cineole 75.78%	Nanocapsule	62.1 ± 1.25 nm	35.5% treatment efficacy at 5.0%		Galli et al. (2018)
<i>R. microplus</i>	<i>Cinnamomum verum</i> , <i>Cuminum cyminum</i> , and <i>Pimenta dioica</i> EOs	Lauraceae, Apiaceae, and Myrtaceae	Cinnamaldehyde 37.77%	Phyto-formulation (micro)	1057 ± 181.7 nm	95.09% treatment efficacy at 10.0%		Lazcano Díaz et al. (2019)
<i>R. microplus</i>	<i>Cinnamomum verum</i> , <i>Cuminum cyminum</i> , and <i>Pimenta dioica</i> EOs	Lauraceae, Apiaceae, and Myrtaceae	Cinnamaldehyde 37.77%	Phyto-formulation (micro)	856.8 ± 216.4 nm	82.11% treatment efficacy at 10.0%		Lazcano Díaz et al. (2019)
<i>R. sanguineus</i>	d-Limonene			Nanoemulsion	258.5 nm	LC <sub>50</sub> by indirect contact on eggs 4.88% LC <sub>50</sub> by indirect contact on larvae 7.82% LC <sub>50</sub> by direct contact on adults 7.28%	AChE inhibition 30.33 ± 2.516% Phoxim at 0.50 µL mL <sup>-1</sup> was 100% effective on all the life stages but showed less AChE inhibition	Ibrahim et al. (2022c)
<i>R. sanguineus</i>	<i>Pelargonium graveolens</i> EO + <i>Origanum majorana</i> EO + thymol	Geraniaceae and Lamiaceae	Citronellol 14.4% and Carvacrol 58.4%	Nanoemulsion	Unknown	73.33 ± 5.773% mortality of adult females 6.265 ± 2.380% reproduction efficacy		Gadelhaq et al. (2024)
<i>R. sanguineus sensu lato</i>	Thymol + eugenol			Micellar dispersion	Unknown	From 5.0 mg mL <sup>-1</sup> by indirect contact 73.32 ± 31.25% mortality on unfed larvae 98.75 ± 3.54% mortality on unfed nymphs 100% mortality on engorged larvae and nymphs		Coelho et al. (2020)
<i>R. sanguineus sensu lato</i>	Thymol + eugenol			Microemulsion	30.94 nm	Reduced number of larvae after 2 d and egg hatching (14.3 ± 20.7%)	Physical stability for 24 months No haematological and biochemical adverse effects on dogs	Monteiro et al. (2021)

Hard tick species: *Hyalomma dromedarii*, *Rhipicephalus annulatus*, *Rhipicephalus australis*, *Rhipicephalus microplus*, and *Rhipicephalus sanguineus*; LC<sub>50</sub> median lethal concentration; RC<sub>50</sub> median repellent concentration; AChE acetylcholinesterase

most accredited toxicity tests on varroa mites follow the complete exposure method (a combination of contact and fumigation and, potentially, oral intake) modified from Lindberg et al. (2000).

### 2.1. Hard ticks (*Ixodida*: *Ixodidae*)

The so-called hard ticks, belonging to the family Ixodidae, include 701 species of blood-feeding ectoparasites of mammals, birds, reptiles, and amphibians worldwide (Benelli et al., 2016). Besides causing direct

Table 2

Effects of metal nanoparticles obtained through green routes from plant extracts or single constituents on hard ticks (Ixodida: Ixodidae).

Tick species	Plant extract	Family	Metal	Particle size	Effect	Notes	Reference
<i>Ha. bispinosa</i>	<i>Musa × paradisiaca</i>	Musaceae	Silver	60–150 nm	LC <sub>50</sub> by indirect contact on larvae 1.87 mg L <sup>-1</sup>		Jayaseelan et al. (2012)
<i>Ha. bispinosa</i>	<i>Euphorbia prostrata</i>	Euphorbiaceae	Silver	25–80 nm	LC <sub>50</sub> by direct contact on adults 2.30 ± 0.23 mg L <sup>-1</sup>	No adverse effects on the non-target species <i>C. dubia</i> and <i>D. magna</i>	Zahir and Rahuman (2012)
<i>Ha. bispinosa</i>	<i>Calotropis gigantea</i>	Apocynaceae	Titanium	160–220 nm	LC <sub>50</sub> by indirect contact on adults 9.15 mg L <sup>-1</sup>		Marimuthu et al. (2013)
<i>Ha. bispinosa</i>	<i>Aegle marmelos</i>	Rutaceae	Copper	40.48–50.4 nm	LC <sub>50</sub> by indirect contact on adults 9.50 mg L <sup>-1</sup>		Kamaraj et al. (2022)
<i>Ha. bispinosa</i>	<i>Mangifera indica</i>	Anacardiaceae	Titanium	30 ± 5 nm	LC <sub>50</sub> by indirect contact on larvae 10.64 mg L <sup>-1</sup>		Rajakumar et al. (2015)
<i>Ha. bispinosa</i>	<i>Atalantia monophylla</i>	Rutaceae	Silver	18–25 nm	LC <sub>50</sub> by indirect contact on larvae 40.25 µg mL <sup>-1</sup>	Minor negative impact on the non-target species <i>A. sulcatus</i> , <i>A. bouvieri</i> , <i>A. mitificus</i> , and <i>C. moluccensis</i>	Elumalai et al. (2022)
<i>Hy. anatolicum</i>	<i>Ocimum canum</i>	Lamiaceae	Silver	25–110 nm	LC <sub>50</sub> by indirect contact on larvae 0.78 mg L <sup>-1</sup>		Jayaseelan and Rahuman (2012)
<i>Hy. anatolicum</i>	<i>Solanum trilobatum</i>	Solanaceae	Titanium	70 nm	LC <sub>50</sub> by indirect contact on larvae 4.11 mg L <sup>-1</sup>		Rajakumar et al. (2014)
<i>Hy. anatolicum</i>	<i>Astragalus sinicus</i>	Fabaceae	Copper	15–75 nm	LC <sub>50</sub> by indirect contact on larvae 11.30 µg mL <sup>-1</sup> LC <sub>50</sub> by direct contact on adult females 23.03 µg mL <sup>-1</sup>	Increased lipid peroxidation and suppressed levels of AChE and antioxidant activity in larvae	Gattan et al. (2023)
<i>Hy. anatolicum</i>	<i>Mangifera indica</i>	Anacardiaceae	Titanium	30 ± 5 nm	Negative effect on the reproductive performance 100% larval repellency for 180 min at 200 µg mL <sup>-1</sup> LC <sub>50</sub> by indirect contact on larvae 12.22 mg L <sup>-1</sup>		Rajakumar et al. (2015)
<i>Hy. dromedarii</i>	<i>Olea europaea</i>	Oleaceae	Nickel	32.94 nm	About 95% adult mortality with 0.06 µg mL <sup>-1</sup> by ingestion		Alghamdi et al. (2024)
<i>Hy. dromedarii</i>	<i>Olea europaea</i>	Oleaceae	Silver	Unknown	About 80% adult mortality with 0.06 µg mL <sup>-1</sup> by ingestion		Alotaibi et al. (2024)
<i>Hy. dromedarii</i>	<i>Lupinus albus</i>	Fabaceae	Silver	25–90 nm	LC <sub>50</sub> by indirect contact on larvae 3.1 µg mL <sup>-1</sup> LC <sub>50</sub> by direct contact on adult females 6.3 µg mL <sup>-1</sup>	Increased oxidative stress and suppressed levels of antioxidant enzymes and AChE Comparable efficacy with deltamethrin	Majeed et al. (2023)
<i>Hy. dromedarii</i>	<i>Melia azedarach</i>	Meliaceae	Nickel	21–35 nm	Negative effect on the reproductive performance LC <sub>50</sub> by direct contact on eggs 5.00 mg L <sup>-1</sup> LC <sub>50</sub> by direct contact on larvae 7.15 mg L <sup>-1</sup> LC <sub>50</sub> by direct contact on engorged nymphs 1.90 mg L <sup>-1</sup>	No mortality on unfed adults Negative effect on the reproductive performance	The oral administration of 500 mg kg <sup>-1</sup> d <sup>-1</sup> for 5 d to healthy adult mice showed a significant increase of white blood cells and haemoglobin and histopathological alterations in the liver and kidneys Abdel-Ghany et al. (2021b)

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Table 2 (continued)

Tick species	Plant extract	Family	Metal	Particle size	Effect	Notes	Reference
<i>Hy. marginatum</i>	<i>Ocimum canum</i>	Lamiaceae	Silver	25–110 nm	LC <sub>50</sub> by indirect contact on larvae 1.00 mg L <sup>-1</sup>		Jayaseelan and Rahuman (2012)
<i>Hy. marginatum</i>	<i>Lawsonia inermis</i>	Lythraceae	Zinc + Silver	20–160 nm	90% adult mortality by contact with 15 mg mL <sup>-1</sup> after 96 h		Shnawa et al. (2023)
<i>Hyalomma</i> spp.	<i>Azadirachta indica</i>	Meliaceae	Zinc	15–25 nm	LC <sub>50</sub> by indirect contact on larvae 3.28 mg L <sup>-1</sup> LC <sub>50</sub> by direct contact on adults 4.76 mg L <sup>-1</sup>		Zaheer et al. (2021)
<i>Hyalomma</i> spp.	<i>Cymbopogon citratus</i>	Poaceae	Zinc	15–25 nm	100% egg mortality from 1.8 mg L <sup>-1</sup> LC <sub>50</sub> by indirect contact on larvae 3.62 mg L <sup>-1</sup> LC <sub>50</sub> by direct contact on adults 4.92 mg L <sup>-1</sup> 100% egg mortality from 2.1 mg L <sup>-1</sup>		Zaheer et al. (2021)
<i>R. microplus</i>	<i>Lobelia leschenaultiana</i>	Campanulaceae	Zinc	20–65 nm	LC <sub>50</sub> by indirect contact on adults 0.0017 mg L <sup>-1</sup>		Banumathi et al. (2016)
<i>R. microplus</i>	<i>Manilkara zapota</i>	Sapotaceae	Silver	70–140 nm	LC <sub>50</sub> by indirect contact on larvae 3.44 mg L <sup>-1</sup>		Rajakumar and Rahuman (2012)
<i>R. microplus</i>	<i>Aegle marmelos</i>	Rutaceae	Copper	40.48–50.4 nm	LC <sub>50</sub> by indirect contact on larvae 4.30 mg L <sup>-1</sup>		Kamaraj et al. (2022)
<i>R. microplus</i>	<i>Calotropis gigantea</i>	Apocynaceae	Titanium	160–220 nm	LC <sub>50</sub> by indirect contact on larvae 5.43 mg L <sup>-1</sup>		Marimuthu et al. (2013)
<i>R. microplus</i>	<i>Momordica charantia</i>	Cucurbitaceae	Zinc	21.32 nm	LC <sub>50</sub> by indirect contact on larvae 6.87 mg L <sup>-1</sup>		Rajiv Gandhi et al. (2017)
<i>R. microplus</i>	<i>Cissus quadrangularis</i>	Vitaceae	Silver	42.46 nm	LC <sub>50</sub> by indirect contact on larvae 7.61 mg L <sup>-1</sup>		Santhoshkumar et al. (2012)
<i>R. microplus</i>	<i>Mimosa pudica</i>	Fabaceae	Silver	25–60 nm	LC <sub>50</sub> by indirect contact on larvae 8.98 mg L <sup>-1</sup>		Marimuthu et al. (2011)
<i>R. microplus</i>	<i>Mangifera indica</i>	Anacardiaceae	Titanium	30 ± 5 nm	LC <sub>50</sub> by indirect contact on larvae 13.21 mg L <sup>-1</sup>		Rajakumar et al. (2015)
<i>R. microplus</i>	2',3'-Dehydrosalannol		Silver	Unknown	LC <sub>50</sub> by indirect contact on larvae 14.42 ppm LC <sub>50</sub> by direct contact on adult females 298.07 ppm IC <sub>50</sub> 37.09 ppm	For deltamethrin: LC <sub>50</sub> on larvae 49.71 ppm, adults 237.87 ppm, and IC <sub>50</sub> 43.78 ppm	Avinash et al. (2017)
<i>R. microplus</i>	Quercetin dihydrate		Silver	Unknown	LC <sub>50</sub> by indirect contact on larvae 22.6 ppm LC <sub>50</sub> by direct contact on adult females 454.13 ppm IC <sub>50</sub> 76.55 ppm	For deltamethrin: LC <sub>50</sub> on larvae 49.71 ppm, adults 237.87 ppm, and IC <sub>50</sub> 43.78 ppm	Avinash et al. (2017)
<i>R. microplus</i>	<i>Azadirachta indica</i>	Meliaceae	Silver	Unknown	LC <sub>50</sub> by indirect contact on larvae 35.40 ppm LC <sub>50</sub> by direct contact on adult females 261.08 ppm IC <sub>50</sub> 8.02 ppm	For deltamethrin: LC <sub>50</sub> on larvae 49.71 ppm, adults 237.87 ppm, and IC <sub>50</sub> 43.78 ppm	Avinash et al. (2017)
<i>R. microplus</i>	<i>Atalantia monophylla</i>	Rutaceae	Silver	18–25 nm	LC <sub>50</sub> by indirect contact on larvae 51.87 µg mL <sup>-1</sup>	Minor negative impact on the non-target species <i>A. sulcatus</i> , <i>A. bouvieri</i> , <i>A. mitificus</i> , and <i>C. moluccensis</i>	Elumalai et al. (2022)
<i>R. sanguineus</i>	<i>Atalantia monophylla</i>	Rutaceae	Silver	18–25 nm	LC <sub>50</sub> by indirect contact on larvae 60.53 µg mL <sup>-1</sup>	Minor negative impact on the non-target species <i>A. sulcatus</i> , <i>A. bouvieri</i> , <i>A. mitificus</i> , and <i>C. moluccensis</i>	Elumalai et al. (2022)

Hard tick species: *Haemaphysalis bispinosa*, *Hyalomma anatolicum*, *Hyalomma dromedarii*, *Hyalomma marginatum*, *Rhipicephalus microplus*, and *Rhipicephalus sanguineus*; non-target species: *Acilius sulcatus*, *Anisops bouvieri*, *Araneus mitificus*, *Ceriodaphnia dubia*, *Cyrtophora moluccensis*, and *Daphnia magna*; LC<sub>50</sub> median lethal concentration; IC<sub>50</sub> median inhibition concentration; AChE acetylcholinesterase

Table 3

Effects of microcapsules loaded with essential oils (EOs), oils, plant extracts, single constituents, or their mixtures on house dust mites (*Dermatophagoides* spp.).

Botanical active ingredient	Family	Main compound	Formulation	Particle size	Effect	Notes	Reference
Clove bud ( <i>Syzygium aromaticum</i> ) EO	Myrtaceae	Eugenol > 75%	Microcapsule	2.9 µm	100% mortality on <i>D. farinae</i> from 20 wt% microcapsules	Grafted on fabric	Kim and Kim (2017)
Eucalyptus ( <i>Eucalyptus</i> spp.) EO	Myrtaceae	1,8-Cineole 75.8%	Microcapsule	1.8 µm	98.7% mortality on <i>D. farinae</i> after 72 h	Grafted on fabric	Kim (2017)
Eugenol			Microcapsule	1.46 µm	96 ± 2.0% mortality on <i>D. farinae</i> from 20 wt% microcapsules	Extruded in fabric	Lee and Kim (2020)
Clove bud ( <i>Syzygium aromaticum</i> ) EO	Myrtaceae	Eugenol 83.66%	Microcapsule	33 µm	93.88% mortality on <i>D. farinae</i> after 72 h		Kim and Sharma (2011)
Red thyme ( <i>Thymus</i> spp.) EO	Lamiaceae	Thymol 54.41%	Microcapsule	21 µm	83.67% mortality on <i>D. farinae</i> after 72 h		Kim and Sharma (2011)
<i>Paeonia</i> × <i>suffruticosa</i> EO	Paeoniaceae	Paeonol 99.05%	Nanoparticle	150 nm	LC <sub>50</sub> by contact 0.19 mg cm <sup>-2</sup> , by fumigation 0.52 mg cm <sup>-3</sup> on <i>D. farinae</i> adults after 24 h	AChE inhibition; SOD increase after 24 h	Teng et al. (2024)
<i>Paeonia</i> × <i>suffruticosa</i> EO	Paeoniaceae	Paeonol 99.05%	Nanoemulsion	300 nm	LC <sub>50</sub> by contact 0.25 mg cm <sup>-2</sup> , by fumigation 0.29 mg cm <sup>-3</sup> on <i>D. farinae</i> adults after 24 h	AChE inhibition; SOD increase after 24 h	Teng et al. (2024)
4,5-dimethylfurfural			Nanoemulsion	161.3 ± 32.3 nm	LD <sub>50</sub> by contact+fumigation on <i>D. farinae</i> 7.24 µg cm <sup>-2</sup>	LD <sub>50</sub> for benzyl benzoate 12.11 µg cm <sup>-2</sup> and for DEET 20.26 µg cm <sup>-2</sup>	Choi et al. (2021)
Neem ( <i>Azadirachta indica</i> ) oil	Meliaceae		Microcapsule	36 ± 0.63 µm	100% mortality on <i>D. pteronyssinus</i> after 16 h from 55% EO	Sprayable formulation	Moeini et al. (2023)
Eugenol			Nanoparticle	2460 nm	92% mortality on <i>D. pteronyssinus</i> after 24 hours with 4.29% eugenol	If applied on fabric by dipping, <i>D. pteronyssinus</i> mortality was 12%	Jarupaiboon et al. (2007)
4,5-Dimethylfurfural			Nanoemulsion	161.3 ± 32.3 nm	LD <sub>50</sub> by contact+fumigation on <i>D. pteronyssinus</i> 6.87 µg cm <sup>-2</sup>	LD <sub>50</sub> for benzyl benzoate 11.09 µg cm <sup>-2</sup> and for DEET 17.40 µg cm <sup>-2</sup>	Choi et al. (2021)
Benzyl benzoate			Microcapsule	3 µm	82.3% repellency for dust mites before washing	Immobilised on fabric	Wei et al. (2016)
<i>Codonopsis pilosula</i> extract + thyme ( <i>Thymus</i> spp.) EO	Campanulaceae and Lamiaceae		Microcapsule	1.611 µm	76.53% repellency for dust mites before washing	Extruded in fabric	Li and Yu (2020)
<i>Isatis tinctoria</i> extract + <i>Pelargonium roseum</i> extract + <i>Mentha</i> × <i>piperita</i> EO	Brassicaceae, Geraniaceae, and Lamiaceae		Microcapsule	Unknown	76.53% repellency for dust mites before washing	Extruded in fabric	Wu et al. (2019)

House dust mite species: *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*; LC<sub>50</sub> median lethal concentration; LD<sub>50</sub> median lethal dose; AChE acetylcholinesterase; SOD superoxide dismutase

blood losses, skin lesions, irritations, anaemia, and allergic reactions, they transmit pathogenic organisms that are agents of acute and chronic human and animal (pets, livestock, wildlife) illnesses (Magnarelli, 2009; Benelli, 2020). The species on which some botanical formulations were tested are the cattle ticks *Rhipicephalus annulatus* Say, *Rhipicephalus australis* Fuller, *Rhipicephalus microplus* Canestrini, and *Haemaphysalis bispinosa* Neumann, the brown dog tick *Rhipicephalus sanguineus* Latreille, the camel tick *Hyalomma dromedarii* Koch, the Mediterranean *Hyalomma marginatum* Koch and Asiatic *Hyalomma anatolicum* Koch ticks, the black-legged tick *Ixodes scapularis* Say, the wood tick *Ixodes ricinus* L., and the lone star tick *Amblyomma americanum* L.

Considering the medical and veterinary relevance of ticks worldwide, there is a plethora of studies regarding the toxicant and/or repellent action of the most varied botanical-based formulations (Tables 1 and 2).

### 2.1.1. Toxicant formulations containing essential oils and other plant extracts

Numerous studies have explored the toxicant properties of botanical-

based formulations against hard ticks, with a prevalence of nano- and microformulations. For instance, a trial by dos Santos et al. (2017) evaluated the efficacy of a nanostructured *Cinnamomum verum* J.Presl. (Lauraceae) EO against *R. microplus* *in vitro* and *in vivo* on dairy cows. The nanoemulsion and nanocapsules containing *C. verum* EO compromised oviposition, even at lower concentrations, compared to pure EO. Similarly, a microemulsion of a *Cymbopogon winterianus* Jowitt ex Bor (Poaceae) EO improved the acaricidal activity against larvae and adults of this same tick species, due to its smaller particle size allowing better penetration through the tick's epicuticle (Chaisri et al., 2019). Likewise, an *Allium sativum* L. (Amaryllidaceae) EO was more toxic to *R. annulatus* adults when nanoemulsioned and caused tick mouthparts degeneration (Abd-Elrahman et al., 2024). Two nanoemulsions derived from *Artemisia herba-alba* Asso (Asteraceae) and *Melia azedarach* L. (Meliaceae) soxhlet extracts were lethal for all the developmental stages of *Hy. dromedarii* and sublethally compromised the reproductive output of engorged females (Abdel-Ghany et al., 2021a). Nanoemulsions can be a promising tool to control resistant pest strains too. Ibrahim et al. (2022a) investigated the acaricidal activity of a *Pelargonium graveolens* (Thunb.) L'Hér.



Table 4

Effects of micro- and nanoformulations loaded with essential oils (EOs), oils, plant extracts, singular constituents, or their mixtures on mite species.

Botanical active ingredient	Family	Main compound	Formulation	Particle size	Effect	Reference
<b>Tyrophagus putrescentiae</b>						
<i>Achillea fragrantissima</i> EO	Asteraceae	cis-Thujone 24.9%	Nanoemulsion	91.3 ± 9.6 nm	LC <sub>50</sub> 4.7 µL L air <sup>-1</sup> by fumigation	Al-Assiuty et al. (2019)
<i>Achillea santolina</i> EO	Asteraceae	Fragranyl acetate 26.1%	Nanoemulsion	104.6 ± 14.1 nm	LC <sub>50</sub> 9.6 µL L air <sup>-1</sup> by fumigation	Al-Assiuty et al. (2019)
<i>Ocimum basilicum</i> EO	Lamiaceae	Methyl eugenol 71.3%	Nanoemulsion	78.5 ± 11.2 nm	LC <sub>50</sub> 2.2 µL L air <sup>-1</sup> by fumigation	Al-Assiuty et al. (2019)
4,5-Dimethylfurfural			Nanoemulsion	161.3 ± 32.3 nm	LD <sub>50</sub> by contact+fumigation 5.59 µg cm <sup>-2</sup> (LD <sub>50</sub> for benzyl benzoate 9.95 µg cm <sup>-2</sup> and for DEET 18.54 µg cm <sup>-2</sup> )	Choi et al. (2021)
<b>Acarus siro</b>						
<i>Mentha longifolia</i> EO	Lamiaceae	Piperitenone oxide 45.1%	Nanoemulsion	150–300 nm	82.2 ± 3.6% adult mortality 65.6 ± 5.0% nymph mortality at 1000 ppm after 7 d (respectively, 73.3 ± 4.7 and 56.7 ± 5.3% mortality with pirimiphos-methyl)	Kavallieratos et al. (2022a)
<i>Trachyspermum ammi</i> EO	Apiaceae	Thymol 62.6%	Nanoemulsion	170.33 ± 2.20 nm	89.4 ± 3.8% adult mortality 86.6 ± 3.0% nymph mortality at 1000 ppm after 7 d (respectively, 68.9 ± 7.5 and 50.0 ± 5.3% mortality with pirimiphos-methyl)	Kavallieratos et al. (2022b)
<i>Pimpinella anisum</i> EO	Apiaceae	(E)-Anethole 96.8%	Nanoemulsion	58.52 ± 0.33 nm	38.1 ± 3.7% adult mortality 30.8 ± 2.5% nymph mortality at 1000 ppm after 7 d (respectively, 68.9 ± 7.5 and 50.0 ± 5.3% mortality with pirimiphos-methyl)	Kavallieratos et al. (2022b)
<b>Sarcoptes scabiei</b>						
<i>Pelargonium maculatum</i> EO	Geraniaceae	Geraniol 47.89% post formulation	Nanoparticle	259 ± 12 nm	Significant <i>S. scabiei</i> mortality	Pérez et al. (2024)
<i>Mentha × piperita</i> EO	Lamiaceae	Menthol 81.37% post formulation	Nanoparticle	381 ± 29 nm	Lower <i>S. scabiei</i> mortality than in the control	Pérez et al. (2024)
Neem ( <i>Azadirachta indica</i> ) oil	Meliaceae		Microemulsion	Unknown	LT <sub>50</sub> by contact 81.75 m on <i>S. scabiei</i> var. <i>cuniculi</i>	Xu et al. (2010)
<b>Dermanyssus gallinae</b>						
Garlic ( <i>Allium</i> spp.) EO + rosehip ( <i>Rosa</i> spp.) EO	Amaryllidaceae and Rosaceae		Silver nanoparticles	5 nm	100% mortality with 1 mL of 75 ppm silver + 1 mL EO after 1 h	Ismail et al. (2020)
Garlic ( <i>Allium</i> spp.) EO + rosehip ( <i>Rosa</i> spp.) EO	Amaryllidaceae and Rosaceae		Magnetite nanoparticles	20 nm	70.33 ± 0.47% mortality with 1 mL of 100 ppm magnetite + 1 mL EO after 1 h	Ismail et al. (2020)
<b>Varroa destructor</b>						
<i>Acantholippia seriphoides</i> EO	Verbenaceae	Thymol 29.2%	Microcapsule	200 µm	99 ± 9.4% mortality with the complete exposure method and 97 ± 11.4% with the evaporation method using 1 g of microcapsules containing 10% (v/v) EO after 72 h	Ruffinengo et al. (2014)
<i>Schinus molle</i> EO	Anacardiaceae	β-Phellandrene 28.3%	Microcapsule	200 µm	54 ± 22.2% mortality with the complete exposure method and 58 ± 18.2% with the evaporation method using 1 g of microcapsules containing 10% (v/v) EO after 72 h	Ruffinengo et al. (2014)
Oregano ( <i>Origanum</i> spp.) EO	Lamiaceae		Microcapsule	Unknown	21.3 ± 3.3% mortality with 60 g of microcapsules containing 10% (w/v) EO	Sabahi et al. (2020)
		Thymol	Nanoemulsion	176 nm	70.45% fall at 25 ppm	Gamal Eldin et al. (2024)

LC<sub>50</sub> medial lethal concentration; LD<sub>50</sub> median lethal dose; LT<sub>50</sub> median lethal time

(Geraniaceae) EO nanoemulsion against deltamethrin-resistant *R. annulatus*. The nanoemulsion showed superior efficacy in both *in vitro* and *in vivo* assays on infested cattle (whose displayed normal clinical signs and appetite and no redness, itching, or hair loss), outperforming the pyrethroid. While these botanical formulations show promise in tick control and host protection, further research is warranted to optimise their effectiveness, safety, and practical application. Other investigations into potential adverse effects on non-target organisms, as observed in studies involving Swiss albino mice, underscore the importance of comprehensive toxicity assessments (Abdel-Ghany et al., 2021a). Indeed, the small particle size of the formulations that facilitates efficient penetration through the pest cuticle could,

concurrently, impact on the non-target host animals.

Some studies have also explored EO formulations without specifying their particle sizes. As an example, de Mello et al. (2014) investigated emulsions containing EOs mixed with various additives against *R. microplus* engorged females. Emulsions containing a *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae) EO showed overall efficacy of 92.47–100% in reducing tick oviposition and larval hatching, while those with a *C. winterianus* EO were less effective (55.51%). Similarly, Ferreira et al. (2018) tested a *S. aromaticum* EO and pure eugenol formulations against *R. microplus* larvae and females. Both treatments caused larval mortality at low concentrations and showed high efficacy against adult females, but with unexpected equal effectiveness between

formulated and unformulated EOs. Lastly, a methanolic extract of *Pouteria gardneriana* (A.DC.) Radlk. (Sapotaceae) leaves encapsulated in chitosan nanoparticles exhibited significant egg mass reduction and female mortality in *R. microplus* (Pereira Barbosa et al., 2016).

Contrasting results were observed by Ibrahim et al. (2022b) when evaluating the acaricidal effects of a *Citrus × limon* (L.) Osbeck (Rutaceae) EO and *M. alternifolia* (Myrtaceae) oil (tea tree oil) and their nanoemulsions on *R. annulatus*. Surprisingly, the pure EO and oil exhibited higher toxicity towards adults and lower egg production than their nanoemulsions. A similar disparity was also reported for a *Eucalyptus globulus* Labill. (Myrtaceae) EO assessed on *R. microplus*, potentially due to the short exposure time during treatment (Galli et al., 2018). Other tea tree oil formulations demonstrated varying efficacy levels under laboratory conditions, with nanocapsules showing complete reproductive inhibition on *R. microplus* females, albeit at different concentrations compared with the diluted oil (Pazinato et al., 2014). However, in an *in vivo* trial on dairy cows, neither the oil nor the nanocapsules were fully effective, suggesting the need for longer contact periods to improve their action (Pazinatto Boito et al., 2016).

#### 2.1.2. Repellent formulations containing essential oils and other plant extracts

Besides their toxicant effects, EO formulations can also exhibit repellent properties when properly formulated and protect the hosts. Nogueira et al. (2020) investigated the repellent activity of a *Pilocarpus spicatus* A. St.-Hil. (Rutaceae) EO nanoemulsion against *R. microplus* larvae. The nanoemulsion showed comparable repellency to the unformulated EO, suggesting preservation of the AIs' characteristics. Similarly, a tea tree oil emulsion and two crystalline microparticle formulations demonstrated repellent effects against *R. australis*, with the faster release microformulation exhibiting the highest repellency (Yim et al., 2016).

Additionally, two botanical-based commercial products, one containing *p*-menthane-3,8-diol and one neem oil, showed repellent effects against larvae of the wood tick *I. ricinus* under field conditions, although their efficacy may not be sufficient for personal anti-tick protection purposes (Garboui et al., 2006). Anyhow, these findings suggest the potential of EO formulations also as repellents against ticks, offering additional strategies for their control and prevention.

#### 2.1.3. Toxicant and repellent formulations containing single phytoconstituents

Various formulations containing single phytoconstituents of EOs or plant extracts have been investigated for their acaricidal and repellent effects. For instance, da Silva Lima et al. (2017 and 2019) micro-encapsulated carvacrol in yeast cell walls, resulting in increased acaricidal activity against *R. microplus* larvae and prolonged efficacy for up to 60 hours compared to unformulated carvacrol. Similarly, a nanoemulsion of d-limonene exhibited acaricidal activity against eggs, larvae, and adults of *R. annulatus* and *R. sanguineus*, although the unformulated d-limonene showed higher toxicity (Ibrahim et al., 2022c). Additionally, a synthetic derivative of spilanthol (an aliphatic alkylamide mainly found in *Acmella oleracea* (L.) R. K. Jansen (Asteraceae) leaves, stems, and flowers) formulated in a nanoemulsion demonstrated superior efficacy in preventing oviposition by *R. microplus* females compared to spilanthol alone (Anholetto et al., 2024).

An encapsulated formulation of nootkatone (a plant-derived sesquiterpene) in a lignin solution showed enhanced acaricidal activity against nymphs of the black-legged tick *I. scapularis* compared to an emulsifiable formulation, with prolonged residual activity and minimal phytotoxicity (Behle et al., 2011). Formulations of nootkatone and carvacrol applied under field conditions also provided short-term control of host-seeking *I. scapularis* and *A. americanum* ticks, with some formulations even improving persistence and efficacy compared to other botanical-based commercial products (Dolan et al., 2009; Jordan et al., 2011; Bharadwaj et al., 2012).

These studies highlight the potential of various formulations containing singular phytoconstituents for tick control, offering options for both acute and residual efficacy in different environmental settings.

#### 2.1.4. Toxicant and repellent formulations containing multiple botanicals

The pursuit of synergistic interactions among different EOs and their constituents holds promise for enhancing acaricidal efficacy against ticks while reducing costs. Gadelhaq et al. (2024) recently formulated a blend of *P. graveolens* and *Origanum majorana* L. (Lamiaceae) EOs with thymol in a simple emulsion, demonstrating good efficacy against *R. sanguineus* ticks in laboratory and field conditions on infested dogs. Similarly, Monteiro et al. (2021) developed a microemulsion containing thymol and eugenol, showing modest potential for controlling larvae of this species on infested dogs (whose showed no haematological and biochemical adverse effects), although further optimization of concentration and formulation strategies may be warranted. Coelho et al. (2020), instead, achieved high tick mortality using a micellar dispersion similarly containing thymol and eugenol, highlighting the importance of formulation type and assay conditions. Lazcano Díaz et al. (2019) explored the synergistic interaction of a ternary mixture of EOs formulated with different surfactants, demonstrating high toxicity against *R. microplus* larvae and adults.

Furthermore, synergies between plant compounds and synthetic acaricides have been proposed as a potential solution to resistance issues and to reduce application rates (Figueiredo et al., 2022). Overall, these studies underscore the diverse approaches being explored to enhance the efficacy of botanical-based formulations against ticks. It is noteworthy that numerous unformulated EOs, plant extracts, and pure compounds showed promising results against various tick species *in vitro* and/or *in vivo*, proving their undeniable potential as alternative tick control agents (Benelli et al., 2016; Banumathi et al., 2017; Nwanade et al., 2020).

#### 2.1.5. Toxicant metal nanoparticles from plant extracts

The use of plant-derived metal NPs offers a novel and eco-friendly approach for tick management (Table 2), leveraging agricultural waste and by-products. Metal NPs obtained through green, biological routes are safer for the environment and human health, less polluting, and more affordable compared to those deriving from chemical and physical approaches (Gupta et al., 2023). In particular, silver (Ag) NPs were extensively studied for their efficacy against various tick species and life stages. The variable outcomes are partly ascribable to the specific sensitivity and partly to the various plants used for their green synthesis. For instance, Alotaibi et al. (2024) synthesised Ag NPs from an aqueous extract of wasted olive (*Olea europaea* L. - Oleaceae) leaves, showing enhanced antifungal and antiparasitic effect when orally administered to *Hy. dromedarii* compared to uncoated Ag NPs and olive extract alone. Majeed et al. (2023) found that Ag NPs from an aqueous seed extract of *Lupinus albus* L. (Fabaceae) effectively controlled larvae and adult females of *Hy. dromedarii* too. Jayaseelan and Rahuman (2012) demonstrated the efficacy of Ag NPs fabricated with an aqueous leaf extract of *Ocimum canum* Sims (Lamiaceae) against *Hy. anatolicum* and *Hy. marginatum* larvae. Furthermore, Ag NPs produced using aqueous extracts of *Musa × paradisiaca* L. (Musaceae) peels or *Euphorbia prostrata* Aiton (Euphorbiaceae) leaves showed promising acaricidal activity against larvae and adults of different tick species (Jayaseelan et al., 2012; Zahir and Rahuman, 2012). Ag NPs from various aqueous plant extracts, such as *Cissus quadrangularis* L. (Vitaceae) stems, *Manilkara zapota* L. P. Royen (Sapotaceae) leaves, and *Mimosa pudica* L. (Fabaceae) leaves, proved efficacy against *R. microplus* larvae (Marimuthu et al., 2011; Rajakumar and Rahuman, 2012; Santhoshkumar et al., 2012). Green-synthesised metal NPs can be a promising tool to control resistant pest strains too. As an example, Avinash et al. (2017) explored the potential of Ag NPs fabricated with an aqueous *Azadirachta indica* A.Juss. (Meliaceae) (neem) leaf extract and two of its components to effectively control deltamethrin-resistant *R. microplus* ticks. Additionally, Elumalai et al.

(2022) investigated Ag NPs obtained using an aqueous leaf extract of *Atalantia monophylla* (Roxb.) A. DC. (Rutaceae) against larvae of the cattle ticks *Ha. bispinosa*, *R. microplus*, and *R. sanguineus*, highlighting their acaricidal potential and minimal impact on some spiders and aquatic species. Indeed, the determination of side effects on non-target organisms is a key issue to understand the applicability of those formulations in operative conditions.

The use of zinc-based NPs, particularly zinc oxide (ZnO), showed promising results in combating ticks. Banumathi et al. (2016) synthesised ZnO NPs from a leaf *Lobelia leschenaultiana* (C.Presl) Skottsb. (Campanulaceae) aqueous extract and found them to be highly effective against *R. microplus* adults. Rajiv Gandhi et al. (2017) explored the acaricidal potential of ZnO NPs fabricated from a *Momordica charantia* L. (Cucurbitaceae) aqueous leaf extract, showing effectiveness against larvae of the same tick species. Similarly, Zaheer et al. (2021) obtained ZnO NPs from ethanolic extracts of neem or *Cymbopogon citratus* (DC.) Stapf (Poaceae) leaves, demonstrating their efficacy against various life stages of *Hyalomma* spp. ticks. Furthermore, Shnawa et al. (2023) developed composite Ag-ZnO NPs using zinc nitrate hexahydrate and silver nitrate in an aqueous leaf extract of *Lawsonia inermis* L. (Lythraceae) and displaying antibacterial, antifungal, and anti-tick properties.

The use of other metal-based NPs, such as copper and titanium, from plant extracts, seems also promising in managing ticks. Kamaraj et al. (2022) fabricated copper oxide (CuO) NPs from an aqueous leaf extract of *Aegle marmelos* L. Corrêa (Rutaceae) and found them to be effective against adults of *Ha. bispinosa* and larvae of *R. microplus*. Similarly, Gattan et al. (2023) synthesised Cu NPs using copper sulfate and an aqueous extract of *Astragalus sinicus* L. (Fabaceae) aerial parts, proving their efficacy against adults and larvae of *Hy. anatolicum* and declined mean number of eggs laid, egg weight, and hatchability. Marimuthu et al. (2013) produced titanium dioxide (TiO<sub>2</sub>) NPs using an aqueous extract of *Calotropis gigantea* (L.) W. T. Aiton (Apocynaceae) flowers, detecting higher efficacy compared to the pure extract against larvae of *R. microplus* and adults of *Ha. bispinosa*. Rajakumar et al. (2014) synthesised TiO<sub>2</sub> NPs from an aqueous leaf extract of *Solanum trilobatum* L. (Solanaceae) and observed significant efficacy against *Hy. anatolicum* larvae. Similarly, Rajakumar et al. (2015) obtained TiO<sub>2</sub> NPs from an aqueous extract of *Mangifera indica* L. (Anacardiaceae) leaves, showing effectiveness against *Ha. bispinosa*, *Hy. anatolicum*, and *R. microplus* larvae.

Alghamdi et al. (2024) made nickel oxide (NiO) NPs from an olive leaf extract, demonstrating significant acaricidal activity against *Hy. dromedarii* adults by ingestion. However, such efficacy was not fully replicated by Abdel-Ghany et al. (2021b). Their NiO NPs from an aqueous extract of *M. azedarach* ripened fruits showed only moderate toxicity against different developmental stages of *Hy. dromedarii*. Indeed, NiO-Melia NPs reduced egg laying and their hatchability, but did not exert significant mortality on unfed adults, possibly due to their highly chitinized cuticle. Additionally, the oral administration of NiO-Melia NPs to mice resulted in histopathological alterations in the liver and kidneys, indicating potential toxicological effects that must be addressed (Abdel-Ghany et al., 2021b).

Overall, these studies highlight the potential of metal-based NPs from plant extracts as effective tools for tick control. Further research is warranted to explore their mechanism of action, safety for all the organisms implicated, and potential application in integrated tick management strategies.

## 2.2. House dust mites (Acariformes: Pyroglyphidae)

House dust mites, such as the European house dust mite *Dermatophagoides pteronyssinus* (Trouessart) and the American house dust mite *Dermatophagoides farinae* (Hughes), despite their common names, are worldwide distributed and account for more than 90% of the total mite population in households (Ahn et al., 2006). These tiny arachnids are potent allergens, known to trigger conditions like atopic dermatitis,

asthma, and allergic rhinitis (Arlian, 2002). Interestingly, they can even cause oral mite anaphylaxis through the ingestion of contaminated flour-based products, particularly pancakes (Sánchez-Borges et al., 2013).

Addressing the challenges posed by dust mites requires an integrated approach, including thorough cleaning, humidity control, and effective chemical treatments. One mechanical solution involves using tightly woven fabrics with pore sizes under 10 µm to prevent mite allergens from penetrating fibres (Vaughan et al., 1999). Additionally, incorporating functional plant agents into household textiles (Table 3), such as mattress covers and pillowcases, can provide chemical control.

Jarupaiboon et al. (2007) developed chitosan nanoparticles loaded with 4.29% eugenol through ionic gelation. While direct contact with these nanoparticles resulted in 92% mortality of *D. pteronyssinus* after 24 hours, exposure to a cotton fabric treated with the same nanoparticles yielded only 12% mortality. This disparity was likely due to the limited release of eugenol from the fabric. Kim and Sharma (2011) created microcapsules of clove bud (*S. aromaticum*) and red thyme (*Thymus* spp.) EOs using coacervation with gelatin, obtaining *in vitro* mortality rates of 93.88 and 83.67% for *D. farinae*, respectively. The efficacy variation was linked to differences in EO composition, with eugenol being the predominant compound in clove bud EO and thymol in red thyme EO.

Wei et al. (2016) developed microcapsules containing a benzyl benzoate core and calcium carbonate shell to repel dust mites, achieving an 82.3% repellent rate before washing the treated nylon fabric and 61.8% after 20 wash cycles. Li and Yu (2020) extruded a viscose fibre containing a combination of a *Codonopsis pilosula* (Franch.) Nannf. (Campanulaceae) extract and thyme (*Thymus* spp.) EO in microcapsules, determining a 76.53% repellent effect against dust mites before washing and 64.65% after 20 wash cycles. These fabric treatments also exhibited antibacterial and antifungal properties. Analogous results were obtained by Wu et al. (2019) when treating a viscose fibre with a mixture of *Isatis tinctoria* L. (Brassicaceae) dried roots, a *Pelargonium roseum* Willd. (Geraniaceae) aqueous extracts, and *Mentha × piperita* L. (Lamiaceae) EO microcapsules.

Clove bud EO-loaded microcapsules were grafted onto nylon fabrics, causing nearly 100% mortality of *D. farinae* after 24 hours (Kim and Kim, 2017). Similarly, cellulose acetate nanofibers containing eugenol-loaded gelatin microcapsules achieved 96 ± 2.0% mortality of the same mite species in 24 hours and maintained 95 ± 1.3% mortality after abrasion (Lee and Kim, 2020). Eucalyptus (*Eucalyptus* spp.) EO-loaded microcapsules on cotton fabrics resulted in 98.7% mortality of *D. farinae* after 72 hours (Kim, 2017).

Beside the fabric treatments, a nanoemulsion and mesoporous silica nanoparticles of a *Paeonia × suffruticosa* Andrews (Paeoniaceae) EO showed promising larvicidal, nymphicidal, and especially adulticidal activities by binding to vital enzymes and prolonged and stable repellency towards *D. farinae* (Teng et al., 2024).

Moreover, Moeini et al. (2023) developed a sprayable miticide system using neem oil encapsulated in poly(D, L-lactic-co-glycolic) acid copolymers and polyvinyl alcohol microparticles, achieving 100% mortality of *D. pteronyssinus* after 16 hours. Choi et al. (2021) tested a nanoemulsion containing 4,5-dimethylfurfural (a structural analogue of a *Valeriana fauriei* Briq. – Caprifoliaceae EO component) against both American and European house dust mites, showing median lethal doses of 6.87 and 7.24 µg cm<sup>-2</sup>, respectively. Kim et al. (2014, 2017) and Perumalsamy et al. (2014) investigated various spray formulations of undefined size containing EOs like *Juniperus oxycedrus* L. (Cupressaceae), *Ocimum basilicum* L. (Lamiaceae), and *Thujopsis dolabrata* var. *honda* Makino (Cupressaceae) against *D. farinae*, causing mortality rates ranging from 87% to 100%. These formulations outperformed permethrin at 2.5 g L<sup>-1</sup>.

Other unformulated EOs, plant extracts, and single constituents or their analogues showed diverse toxicity degrees towards house dust mites, obviously with slightly different susceptibility based on the species-compound interaction, as shown in the review by Ahn et al.

(2006). While metal NPs have also been tested, only chemical approaches for their synthesis were employed.

### 2.3. Mould or cheese mite (*Sarcoptiformes: Acaridae*)

*Tyrophagus putrescentiae* (Schrank), commonly known as the mould or cheese mite, is a cosmopolitan stored-product mite notorious for infesting a variety of food products, including processed and unprocessed pork, dry-cured hams, cheese, grains, nuts, and other high-fat and high-protein foods (Hill, 2003). Its presence poses significant challenges, as it can cause allergies, contact dermatitis, and asthma in workers involved in food processing, ageing, and storage, and it has been implicated in systemic anaphylaxis in individuals consuming mite-contaminated food (Sánchez-Borges et al., 2013). To ensure food safety, reduce economic losses, and safeguard the well-being of both employees and consumers, effective management strategies are essential.

Recent approaches to managing *T. putrescentiae* focus on using food-grade protective coatings for dry-cured hams, with propylene glycol (PG) being a prominent ingredient (Zhao et al., 2016; Campbell et al., 2017). Building upon this, Campbell et al. (2020) used polyester nets infused with a mixture of PG, propylene glycol alginate, and carrageenan to wrap and hang hams in commercial ageing facilities, successfully inhibiting mould mite growth over an 11-week period. Expanding on this concept, we suggest integrating EOs into PG coatings, leveraging the Generally Recognized As Safe (GRAS) status of several EOs listed in the Code of Federal Regulations of the United States Food and Drug Administration (FDA, 2022) and their widespread use as flavouring agents. Culinary herbs and spices such as bay, capsicum, fennel, nutmeg, pepper, and sage could serve as promising candidates for this purpose. However, conducting sensory evaluations (Farina et al., 2022) to assess the impact of these EO-loaded coatings on meat flavour, texture, and moisture retention, as previously done for other coatings (Zhao et al., 2016; Campbell et al., 2017), would be imperative. Formulations containing PG and EOs are already known in perfumery and cosmetics but even against insect pests (Kurniawan et al., 2022; Rasidah et al., 2022) and the coconut mite (Acari: Eriophyidae) (Costa Barreto et al., 2022).

Regarding the botanical formulations (Table 4), Al-Assiuty et al. (2019) explored the acaricidal potential of EOs extracted from *Achillea fragrantissima* (Forssk.) Sch. Bip., *A. santolina* Sm. (Asteraceae), and *O. basilicum* (Lamiaceae), along with their nanoemulsions, against *T. putrescentiae*. Among these, the *O. basilicum* EO exhibited the highest toxicity, followed by *A. fragrantissima* and *A. santolina*. Nanoemulsions of these EOs showed even greater efficacy, consistent with the principle that smaller particle sizes enhance acaricidal action. Choi et al. (2021) further demonstrated the potency of 4,5-dimethylfurfural, a structural analogue of a *V. fauriei* EO component, against this mite species detecting remarkable acaricidal activity, surpassing that of pure *V. fauriei* EO, so underscoring the benefits of nanometric particle size and improved water solubility and bioavailability.

In addition, a formulation containing a *T. dolabrata* var. *hondae* oil was effective against *T. putrescentiae*, providing mortality rates ranging from 79 ± 3.1 to 100% (Kim et al., 2014). Notably, the high toxicity of *Angelica koreana* Maxim. (Apiaceae) root and *Cinnamomum cassia* (L.) D. Don (Lauraceae) bark pure EOs and some of their constituents to *T. putrescentiae* (Oh et al., 2012; Kang and Lee, 2018) suggests their potential for incorporation into nanoemulsions or PG coatings, thereby enhancing their efficacy as mite control agents.

### 2.4. Flour mite (*Sarcoptiformes: Acaridae*)

*Acarus siro* L., commonly known as the flour mite, is a ubiquitous stored-product pest primarily infesting cereals, flour, bran, dried seeds, fruits, vegetables, meat, shrimps, and milk (Hagstrum et al., 2013). Found in farms, flour mills, and grain warehouses, it poses risks of

dermal irritations and allergies to facility users (Arlian, 2002) and can also be a nuisance in domestic settings (Ahn et al., 2006).

As detailed in Table 4, Kavallieratos et al. (2022a) investigated the acaricidal properties of a *Mentha longifolia* (L.) (Lamiaceae) EO and its nanoemulsion against the flour mite. Treating durum wheat with 1000 ppm of EO and its nanoemulsion resulted in comparable adult and nymphal mortality rates. Although the toxicity levels were not statistically different, it is important to note that the concentration of the *M. longifolia* EO in the nanoemulsion was ten times lower than that of pure EO when working with ppm. In a similar setting, Kavallieratos et al. (2022b) evaluated nanoemulsions containing Apiaceae EOs against *A. siro* adults and nymphs. A *Trachyspermum ammi* (L.) EO exhibited superior efficacy and even outperformed the *M. longifolia* EO, particularly considering its lower concentration in the nanoemulsion (8% vs. 10%). Notably, all the pure EOs and nanoemulsions tested caused higher flour mite mortality than the positive control pirimiphos-methyl at label rate (Kavallieratos et al., 2022 a,b). Although these assays were conducted on treated grains, reducing *A. siro* infestations in working facilities could mitigate occupational allergic reactions.

Furthermore, formulating the most effective EOs into fabrics used for work cloths and uniforms, similar to the approaches seen for dust mite control (paragraph 2.2), could further enhance employees' health and safety.

### 2.5. Itch mite (*Acarina: Sarcoptidae*)

Sarcoptic mange, caused by various strains of the itch mite *Sarcoptes scabiei* (L.) depending on the host species, is a highly contagious and itchy skin disease with significant sanitary implications, affecting over 150 mammal species worldwide (Moroni et al., 2022). Female mites actively burrow into the *stratum corneum*, creating tunnels where they live, feed, defecate, and lay eggs (Sharaf, 2024). In livestock farming, strains such as *S. scabiei* var. *cuniculi* in rabbits, *S. scabiei* var. *ovis* in sheep, and *S. scabiei* var. *suis* in pigs can cause substantial weight loss and reproductive issues. Additionally, skin lesions resulting from infestations can lead to secondary bacterial or fungal infections (Niedringhaus et al., 2019). In humans, particularly children and the elderly, infestations of *S. scabiei* var. *hominis* cause scabies, characterised by pruritic lesions, nodules, bullae, or crusts (Sharaf, 2024).

Initial *in vitro* trials evaluated the promising acaricidal properties of neem oil and two neem extracts on *S. scabiei* var. *cuniculi* larvae (Du et al., 2008). Subsequently, as outlined in Table 4, Xu et al. (2010) developed a microemulsion (size not specified) containing 10% neem oil, an emulsifier system composed of polysorbate 80 + sodium dodecyl benzene sulfonate (SDBS), and water. This microemulsion achieved a median lethal time by contact of 81.75 min for *S. scabiei* var. *cuniculi*. In contrast, the effect of a 10% neem oil aqueous emulsion and 10% paraffinic neem oil solution resulted in higher LT<sub>50</sub> values (Xu et al., 2010). As the control microemulsion lacking the oil showed an LT<sub>50</sub> of 89.07 min, the authors inferred that SDBS might possess acaricidal activity, which was also observed by Zang et al. (2005) when testing a 2.0% SDBS cream against human hair follicle mites (*Demodex* spp.).

An aqueous-methanol extract of neem seed kernels formulated at 20% with vaseline to create an ointment was applied *in vivo* to sheep with sarcoptic mange caused by *S. scabiei* var. *ovis*. Under field conditions, after 16 days, the neem-based ointment completely eradicated the mite infestation, whereas the positive control ivermectin at 200 µg kg<sup>-1</sup> required 10 days for that; after 20 days, clinical mange was resolved (compared to 16 days with ivermectin). Although the response to ivermectin was quicker, the efficacy of the neem-based ointment was comparable (Tabassam et al., 2008). Nevertheless, we speculate that a different formulation, such as a nanoemulsion with smaller particle sizes, could further enhance the bioactivity of this potent botanical extract. Indeed, nanoparticles loaded with a *Pelargonium maculatum* G. Don (Geraniaceae) EO significantly reduced the survival of *S. scabiei* mites collected from an infested ibex (Pérez et al., 2024), but additional

formulations are indispensable.

## 2.6. Poultry red mite (*Acarina: Dermanyssidae*)

*Dermanyssus gallinae* (De Geer), the poultry red mite, stands as the most harmful ectoparasitic mite affecting poultry globally. Preferring to feed on the blood of laying hens during the night, it remains concealed in cracks and crevices near chicken coops during the day. This behaviour results in physiological harm, stress, subacute anaemia, weight loss, irritations, and diminished egg production and size (Hwang, 2023). Moreover, this mite species serves as a potential reservoir or vector for numerous bacterial and viral pathogens affecting poultry (Valiente Moro et al., 2009). Due to its broad host range, including mammals such as dogs, horses, rodents, and even humans, the poultry red mite poses a significant threat. In poultry facilities, it can cause occupational dermatitis, known as gamasoidosis, along with erythematous eruptions and urticaria among farmers, technicians, and veterinarians (Cafiero et al., 2019).

Following a laboratory screening, a formulation comprising 20% neem oil in ethoxylated castor oil proved being effective in impregnating cardboard traps deployed within poultry houses, leading to a significant reduction in red mite populations (Lundh et al., 2005). Similarly, a patented formulation containing 20% neem oil achieved remarkable success by reducing the poultry red mite presence by 94.65–99.80% in heavily infested laying hen farms (Camarda et al., 2018).

As indicated in Table 4, Ag and magnetite (MA) nanocomposite particles were chemically synthesised by Ismail et al. (2020) and subsequently combined with a commercial supplement based on EOs (garlic and rosehip). Upon spraying, 100% mortality of *D. gallinae* was achieved with Ag-EO after 1 hour and  $70.33 \pm 0.47\%$  mortality with MA-EO. However, after 2 hours, both treatments reached total mortality. Despite the different time of action, authors recommend employing MA-nanocomposites due to their minimal human toxicity (Ismail et al., 2020).

While several other essential oils were promising against *D. gallinae* (Hwang, 2023), no author has explored botanical nano- or micro-formulations or nanoparticles derived from environmentally friendly methods for controlling this mite yet.

## 2.7. Varroa mite (*Mesostigmata: Varroidae*)

*Varroa destructor* Anderson & Trueman, formerly known as *Varroa jacobsoni* Oudemans until 2000 and commonly referred to as the varroa mite, stands as the primary ectoparasitic mite affecting honeybees, including *Apis mellifera* L. and *Apis cerana* Fabricius (Hymenoptera: Apidae). This species primarily feeds on the fat body of honeybee larvae, pupae, and adults (Ramsey et al., 2019), leading to colony weakening, immunosuppression, and transmission of pathogens, notably the deformed wing virus (Lester et al., 2022). Beekeepers frequently employ various EOs (e.g., clove, eucalyptus, oregano), their individual constituents (e.g., thymol, menthol), and organic acids (e.g., formic acid, oxalic acid) to combat varroasis in the apiaries, with numerous other substances undergoing laboratory and field studies over the years. Several reviews on the topic are available (Umpiérrez et al., 2011; Abou-Shaara, 2014; Aglagane et al., 2021; Bava et al., 2023). These botanical AIs often act as fumigants against the varroa mite, prompting researchers to develop various formulations in different matrices (e.g., starch, sugar, mineral oil, glycerin, Arabic rubber) and dispensers to prolong or regulate their release duration.

Glenn et al. (2007) utilised starch to encapsulate 2-heptanone, a ketone naturally secreted by honeybees and effective at high concentrations against mites (Erickson et al., 2005), also found in clove and cinnamon bark EOs. An aqueous starch gel containing glycerol and three times its weight in 2-heptanone released 50% of the ketone in approximately 13 days. While the target time for effective control is 20 days, laminating the gel with poly(vinyl alcohol) could potentially extend its

duration. Additionally, approximately 18% of 2-heptanone was encapsulated in a polycaprolactone matrix through extrusion, but the controlled release of the active ingredient was unsatisfactory (Glenn et al., 2007).

Sabahi et al. (2017) aimed to evaluate the varroacidal efficacy of three formulated natural compounds using three application methods in hive with slow, rapid, or continuous release over a 4-week period. They employed a 2% oxalic acid solution in sugary syrup to soak corrugated cardboard for slow release, a 7% solution of a mixture of oregano (*Origanum* spp.) and clove EOs in ethanol, water, and mineral oil on adsorbent pads for rapid release, and oregano EO in two electric vaporizers for continuous release. The latter exhibited the highest efficacy rate ( $97.4 \pm 0.68\%$  fallen mites), but despite the continuous release, the EO's evaporation rate was four times higher in the first two weeks than in the last two. Applications of oregano and clove EOs on pads showed the lowest acaricidal effect alongside the highest bee mortality ( $70.22 \pm 17.05\%$ ). It is evident that different formulations and delivery systems can significantly impact the outcome of the treatments. A subsequent investigation by Sabahi et al. (2020) assessed and compared the toxicity of six dry and wet formulations of oxalic acid, oregano EO, and thymol against varroa mites in beehives during fall season. Dry and wet thymol demonstrated the highest activity (over 90% mortality), while, unexpectedly, oregano EO microcapsules in  $\beta$ -cyclodextrin (Table 4) exhibited the least effectiveness ( $21.3 \pm 3.3\%$  mortality). Although microcapsules were intended to prolong the EO activity by stabilising and preventing the evaporation and oxidation of its compounds, the release rate was too low, possibly due to relatively cold temperatures in the field, warranting further trials to elucidate this unexpected outcome. Conversely, glycerin delayed the release of compounds within the hive to appropriate rates (Sabahi et al., 2020). It must be noted that thymol often leave residues in honey and beeswax (Floris et al., 2004; Serra Bonvehí et al., 2015) that might impact on the phototaxis and nervous system of honeybees (Bergougnotx et al., 2012; Glavan et al., 2020), but it is, anyhow, safe for human consumption.

Still with the aim of prolonging their release, Ruffinengo et al. (2014) microencapsulated *Acantholippia seriphoides* (A.Gray) Moldenke (Verbenaceae) and *Schinus molle* L. (Anacardiaceae) EOs in Arabic rubber (Table 4) after a preliminary screening on nine unformulated EOs (Ruffinengo et al., 2005). Microcapsules of the *A. seriphoides* EO were more toxic towards *V. destructor* than those obtained from the *S. molle* EO (more than 97% mortality vs. more than 54% after 72 hours with a 1 g dose) both through the complete exposure method (contact and fumigation at the same time) and evaporation only. Anyhow, the complete exposure method caused  $87 \pm 6.5\%$  and  $42 \pm 10\%$  honeybee mortality with *A. seriphoides* and *S. molle*, respectively, probably due to the cloud of EO powder that was easily inhaled by the honeybees. Even the exposure to the EOs vapours was quite toxic to honeybees (Ruffinengo et al., 2014).

An alternative approach to managing *V. destructor* involved using  $\beta$ -cyclodextrin particles containing thymol, carvacrol, or oregano EO dissolved in a sucrose solution and administered to honeybees as a feeding syrup. The objective was to deliver these monoterpenes into the midgut of honeybees and subsequently to the hemolymph to reach the mites during feeding. Thymol and carvacrol were, indeed, detected at millimolar levels in the gut and hemolymph of treated honeybees without toxicity, although no specific trials involving the mite were conducted (Leblanc et al., 2008). A thymol nanoemulsion mixed with candy (made of sugar, yeast, and water) at a concentration of 25 ppm was used by Gamal Eldin et al. (2024) to feed honeybee colonies under field conditions and caused a mean 70.45% mite fall regardless the temperature and increased pollen consumption, thus indicating a probable enhanced colony immunity. A similar control over the mite was obtained with unformulated thymol but employing a four-fold concentration (100 ppm) that is, therefore, more expensive and less palatable to honeybees.

Protecting honeybees from parasitic mites using botanical-based

acaricides is still a challenge due to the similarities between target and non-target species. Certain acaricides based on botanicals and effective against *V. destructor* are, indeed, not selective towards honeybee workers, queen, larvae, and/or brood (Melathopoulos et al., 2000; Conti et al., 2020). In addition to the acute toxicity, it has long been known that EOs or their compounds can have adverse effects on honeybee colonies, such as irritability, robbing, absconding, and hive abandonment especially when applied at high temperatures (Imdorf et al., 1999).

### 3. Future challenges and conclusions

Concerning the toxicant and repellent action of botanical-based nano- and microformulations towards Acari species of medical and veterinary relevance, the most numerous efforts have been surely made to control hard ticks (Ixodidae), especially *R. microplus*. In detail, the newest applications range from *in vitro* and *in vivo* applications of nano- and microemulsions and nano- and microcapsules loaded with EOs, some of their pure phytoconstituents, or their mixtures to metallic (mainly Ag) NPs obtained through green, biological routes from diverse plant extracts. While most studies have demonstrated enhanced efficacy and prolonged action of botanical AIs when formulated, a few challenges remain, attributed to synthesis technologies, storage conditions, and/or exposure times.

Building upon the successes in tick control, we feel up to suggesting similar solutions to manage, for instance, soft ticks (Argasidae) that have never been contemplated before as target pests in such screenings. Furthermore, the control of the mites mentioned in this review could surely benefit from the experience in the field acquired working with ticks and *vice versa*, but always paying attention to the species-specific sensitivity when selecting the proper concentration/dose. For instance, fabric botanical-based treatments effective against house dust mites could be adapted for use in tick-infested garments, like socks and stockings, or in work cloths and uniforms to combat mite infestations, such as those of flour mites. Expanding the repertoire of tested formulations, the PG-infused nets used to wrap and hang hams during ageing should benefit from the incorporation of FDA-approved food-grade EOs or pure compounds, especially in nano- or microformulations. The metallic NPs synthesised from plant extracts and tested, so far, only on hard ticks, could be helpful for mite management too and substitute the less eco-friendly samples obtained from chemical and physical routes. In any case, even if metallic NPs proved to have a minor negative impact on some non target species, they could, instead, be dangerous for honeybees, if we suppose their use against varroa mites or because of environmental accumulation deriving from other applications (Abou-Shaara et al., 2020).

Unfortunately, against the mites *S. scabiei* and *V. destructor*, even if subjected to numerous toxicity trials involving unformulated EOs, plant extracts, and some of their pure compounds, nano- and microformulations have been scarcely tested so far. The development of effective formulations to control *V. destructor* becomes increasingly important, especially in the context of the ongoing climate change, which may affect the efficacy and applicability of commercial products derived from botanical extracts already being problematic at high temperatures. Indeed, new botanical-based formulations capable of mitigating these side effects are strategic for increasing sustainable varroa mite control and reducing singular honeybees and colony loss.

More generally, besides the limited research efforts on specific formulations and/or pests, we report scarce uniformity in toxicological and repellence trials. Even if the evaluation methodology is often similar, some basic parameters, such as the exposure time to the toxicant AI, are too variable. The unification of investigation methodology and adoption of standardised operating procedures, as requested, for instance, when evaluating the susceptibility of adult mosquitoes to insecticides, would facilitate data interpretation and guide future research directions.

At present, only few botanical-based nanoformulations against Acari are available on the market as ready-to-dilute preparations. One

example is NATURA Rock Effect NEW RTD (manufactured by Agro CS, a. s., Czech Republic), a microemulsion of a *Pongamia pinnata* (L.) Pierre (Fabaceae) EO also suitable for the two-spotted spider mite *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae) management. As there is already a plethora of studies regarding the application of various AIs of botanical origin as toxicants or repellents towards the Acari pests here reviewed, the bases for future research are surely not lacking. What is necessary to improve is the synthesis technologies and storage conditions of the different formulations while scaling up their production to achieve lower prices and wider distribution of such new replacements to the overused and non-sustainable synthetic acaricides. A further strategy to reduce the costs should consider the employment of EOs extracted from already widespread crops. Among these, we can surely include *S. aromaticum*, *Thymus* spp., and *Origanum* spp., common aromatic plants greatly appreciated as food flavourings, medicinal treatments, and anti-tick and anti-mite agents as here documented. Still for medicine and also insect pest control, *A. indica* (in Asia) and *M. alternifolia* (mainly in Australia) are industrially grown too.

In conclusion, the management of mite and tick infestations represents a complex and multifaceted endeavour, requiring interdisciplinary collaboration and ongoing innovation. By addressing future challenges head-on and leveraging advances in science and technology, we can develop more sustainable and effective solutions, ultimately safeguarding the health and well-being of both humans and animals alike.

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### CRedit authorship contribution statement

**Priscilla Farina:** Literature analysis, Writing – original draft, Writing – review & editing. **Giulia Giunti:** Literature analysis, Writing – review & editing. **Orlando Campolo:** Literature analysis, Writing – review & editing. **Filippo Maggi:** Literature analysis, Writing – review & editing. **Renato Ricciardi:** Literature analysis, Writing – review & editing. **Andrea Lucchi:** Literature analysis, Writing – review & editing. **Angelo Canale:** Literature analysis, Writing – review & editing. **Roman Pavela:** Literature analysis, Writing – review & editing. **Raul Narciso C. Guedes:** Literature analysis, Writing – review & editing. **Nicolas Desneux:** Literature analysis, Writing – review & editing. **Giovanni Benelli:** Conceptualization, Literature analysis, Writing – review & editing.

### Declaration of Competing Interest

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### Data availability

This is an Invited Review

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