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RESEARCH

# Common yarrow (*Achillea millefolium*) essential oil and main components as potential repellents and acaricides against *Ixodes scapularis* and *Dermacentor variabilis* (Acari: Ixodidae) ticks

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**Abstract**
Repellent and acaricidal activities of essential oils (EO) extracted from common yarrow (*Achillea millefolium* L.) and main chemical components were evaluated against *Ixodes scapularis* and *Dermacentor variabilis* adult ticks and nymphs. Flowers and leaves were collected from two locations, Harvest Moon trail (HMT) and Port Williams (PW) in Nova Scotia (Canada), and EO were extracted via hydro-distillation. Samples were analyzed using GC-MS, and differences in chemical composition and quantity of compounds detected were reported in relation to the collection site and plant parts. EO were both rich in germacrene D (HMT EO 21.5 ± 1.31% wt; PW EO 25.5 ± 0.76% wt); however, HMT flower EO has a higher concentration of camphor (9.9 ± 0.08% wt) compared to PW flower EO (3.0 ± 0.01% wt). Significant acaricidal activity was reported against *I. scapularis* adult ticks, particularly for HMT flower EO with a $LD_{50}$ of 2.4% v/v (95% confidence interval = 1.74–3.35) at 24 h post-exposure. Germacrene D had the lowest $LD_{50}$ of 2.0% v/v (95% CI 1.45–2.58) among the four compounds after 7 days. No significant acaricidal effect was observed on *D. variabilis* adult ticks. Yarrow PW flower EO exerted repellent activity towards *I. scapularis* nymphs (100% repellency up to 30 min); however, repellency significantly declined over time. Yarrow EO exert promising acaricidal and repellent properties, that may be used to manage *Ixodes* ticks and the diseases they vector.

**Keywords** Ixodidae · Essential oils · Repellent · Acaracide · Camphor · Germacrene D

## Introduction

Ticks (Acari: Ixodidae) are blood-feeding, parasitic arthropods and are known to vector several zoonotic diseases (Wikel 2018). In North America, species such as the blacklegged tick, *Ixodes scapularis* Say, and the American dog tick, *Dermacentor variabilis* (Say), are commonly found in wooded, humid areas and pose a serious public and domestic animal

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health concern. This is due to the diverse array of pathogens which are transmitted through a bite of a tick from either of these species (Sonenshine 2018).

*Ixodes scapularis* ticks are known to transmit several infectious agents (identified as of 2017) which cause illness in humans (Wikel 2018). This includes bacterial agents that cause Lyme disease, human granulocytic anaplasmosis, tick-borne relapsing fever, human babesiosis, ehrlichiosis, as well as the viral agent that causes Powassan illness, and the protozoan parasite *Babesia microti* (Eisen and Eisen 2018). Among this list, Lyme disease caused by *Borrelia burgdorferi* is the most common vector-borne illness in North America and is of the most concern due to its high level of transmissibility (Radolf et al. 2021). A bite from *D. variabilis* is also of concern, due to the potential transmission of the bacterial agent *Rickettsia rickettsii* which causes the most lethal vector-borne disease in the USA, Rocky Mountain Spotted Fever (Masters et al. 2003). Transmission of tick-borne illnesses is on the rise due to widespread warming climates (Randolph 2010). This allows for a longer active tick season causing an increase in tick abundance and increased land area of tick establishment (Bouchard et al. 2019).

A promising approach to reduce disease transmission is the prevention of tick bites by protecting humans through the application of repellents and frequent thorough tick checks (Government of Nova Scotia 2021). Preventing tick bites and controlling the tick population will require innovative solutions. Traditionally, the control of ticks is performed using commercially available tick repellents and acaricides based on synthetic chemicals (Cisak et al. 2012). However, negative impacts of synthetic products such as pollution and bioaccumulation (Roy et al. 2017) together with the development of resistance by the target pest (Ravindran et al. 2018), has led to the search for safer methods without compromising efficacy (Nwanade et al. 2020). Tick repellents and acaricidal products based on botanical active ingredients may provide a safer alternative to synthetic compounds. Essential oils and other natural products have been reported to have valuable repellent and acaricidal properties and they can be an environmentally safer option with low human and animal toxicity (Koul et al. 2008). Based on studies looking at the toxicity of these naturally sourced compounds, most essential oils pose no remarkable risk to fish or mammals, making them more appealing to synthetic repellents (Koul et al. 2008). The past decades have seen the rise of research focusing on the investigation of bioactivity from botanical material, specifically essential oils as they have been classified as GRAS (Generally Recognized As Safe) with potential positive environmental effect over synthetic compounds (Tripathi and Mishra 2016; Isman 2016).

Plant materials provide a rich reservoir of volatile active ingredients that have shown to be repellent and acaricidal against ticks and other acarines (Faraone et al. 2020; Light et al. 2021; Wang et al. 2022). Although the mode of action of such compounds is still under investigation (Selles et al. 2021), the observed activity has been often associated to the presence of specific terpenes and terpenoids (Adamo et al. 2022; Gross et al. 2017). Several essential oils and isolated terpenes have shown to exert inhibitory activity on acetylcholinesterase: $\beta$-phellandrene, limonene, $\alpha$-pinene and $\beta$-pinene, carvacrol, thymol, menthol, and menthone to name a few (dos Santos Cardoso et al. 2020; Anderson and Coats 2012; Arafa et al. 2020).

An interesting source of natural products with promising bioactivity is the common yarrow (*Achillea millefolium* L.), a plant that is found in abundance in the temperate and boreal regions of the Northern hemisphere. Yarrow has valuable antimicrobial, anti-inflammatory, and anti-fungal properties (Villalva et al. 2022; Bashir et al. 2022) and it has been used as a natural resource for repelling pests (Tampe et al. 2015; Jaenson et al. 2006). Additionally, the essential oils from yarrow have shown to have repellent and acaricidal properties

against other acarines, including repellency against Varroa mite (*Varroa destructor*), a parasite that attacks and feeds on honey bees (Light et al. 2021), and acaricidal activity against two-spotted spider mite (*Tetranychus urticae*), an important agricultural pest (Ahmadi et al. 2018; Ebadollahi et al. 2016).

In this work, the repellent and acaricidal properties of essential oils and their main components extracted from the flowers and the leaves of yarrow plants collected from two locations were evaluated on two medically important tick species, the black-legged tick (*I. scapularis*) and the American dog tick (*D. variabilis*).

## Materials and methods

### Chemicals

All chemicals and chemical standards were either purchased from Sigma-Aldrich (Oakville, Ontario, Canada) or kindly provided by Dr. Kirk Hillier (Biology Department, Acadia University). CAS numbers and purity of chemicals used in this study are reported in the supplementary material (Table S2).

### Yarrow collection

Common yarrow was collected in the summer (July-August) of 2021 along the Harvest Moon Trailway (45° 05′ 36″ N, 64° 21′ 30″ W) (hereafter HMT) and dyke-lands (45° 06′ 03″ N, 64° 20′ 40″ W) in Wolfville (NS, Canada) and along roadways in Port Williams (45° 07′ 00″ N, 64° 23′ 51″ W) (NS, Canada) (hereafter PW). Plants were identified using Newcomb (1989) and collected in full bloom, as this stage is characterized by a higher concentration of essential oil (Light et al. 2021). Fresh plant material was separated according to plant parts (flowers, leaves, and stems). Flowers and leaves were stored in Ziploc freezer bags (S.C. Johnson & Son, Varennes, Quebec, Canada) at −20 ºC until further use. Plant material was also separated based on location and date of collection.

### Essential oil extraction and chemical characterization

Essential oils were extracted according to a conventional hydro-distillation method using a Clevenger-type apparatus as previously described (Wang et al. 2022). Plant material was removed from the freezer and ground using a food processor (Black + Decker, Baltimore, MD, USA). Plant material was weighed prior extraction to determine the extraction yield. After extraction carried out for 3 h, essential oils were collected, dried with anhydrous sodium sulfate, and filtered. The recovered oil was weighed and stored in vials at −20 ºC for further use.

Essential oil samples were analyzed via gas chromatography-mass spectrometry (GC–MS) to determine composition. Samples were prepared for injection into a Bruker 456 GC (Bruker Chemical Analysis, Goes, The Netherlands) at a concentration of 50 ng/µL (hexane was used as solvent). One µL of each sample was injected using a Maestro Autosampler (Gerstel, Mülheim an der Ruhr, Deutschland) at 250 ºC with a 1:5 split ratio. Helium was used as the carrier gas at 1 mL/min flow rate through a (5%-phenyl)-methylpolysiloxane DB-5 capillary column (Agilent, Santa Clara, CA, USA). The oven was initially

set to 50 ºC and held for 5 min; then the temperature was increased to 220 ºC at a rate of 7 ºC/min for 29.9 min, and finally increased to 280 ºC at a rate of 30 ºC/min. Samples were analyzed by a Scion 456 MS (Scion Instruments, Livingston, UK).

Qualitative analysis of yarrow EOs (from leaves and flowers collected along HMT and PW) were completed by comparing the retention times (RT) and MS fragmentations of chemical standards with corresponding peaks present within the essential oil. NIST database (NIST/EPA/NIH Mass Spectral Library v.2.0 g 2021, Scion Instruments) was used to search for compounds that presented no match with available chemical standards.

Quantitative analyses were carried out on the HMT and PW yarrow flower EO using an internal calibration method. Data were collected in selected ion monitoring (SIM) mode using the same method previously described for the qualitative analysis. Eugenol and linalool were used as internal standards (IS) and added to selected chemical standards and the unknowns. Standards (camphor, b-pinene, eucalyptol, and germacrene D) at 160, 80, 50, 20, 10, and 5 ng/mL concentrations were prepared in hexanes by a serial dilution. Solutions were injected 6× and the calibration curves were made by plotting the average peak area from each standard peak divided by the average peak area from the internal standard peak against the concentration of each standard. Concentrations of these compounds in the yarrow standard were found in ng/mL and were then converted to wt%.

## Ticks

Ticks were purchased from the Tick Rearing Facility Laboratory at Oklahoma State University (Stillwater, OK, USA). Ticks were kept in containers lined with moistened Kimwipe (Fisher Scientific, Burlington, ON, Canada) and stored in the dark at 4 ºC. Prior to the beginning of bioassays, ticks were removed from the refrigerator and left at room temperature (20–22 °C) for at least 20 min for acclimatation. Naïve, non-engorged adult female *D. variabilis* and *I. scapularis* were used for adult immersion tests to determine the acaricidal activity. Naïve, non-engorged nymphal *D. variabilis* and *I. scapularis* were used for horizontal repellency tests. Ticks used in all bioassays were not older than 5 months from the last molt.

## Acaricidal bioassay

Acaricidal bioassays were performed in the Innovation Pavilion Laboratory, Acadia University (Wolfville). To test the acaricidal activity of yarrow flower EO, we used the adult immersion test (AIT) performed according to Drummond et al. (1973). Five unfed active adult female ticks of the same species were fully immersed for 1 min in a 4-mL vial containing the tested solution. Ticks were removed and placed onto a moist filter paper-lined inside a plastic 9-cm-diameter Petri plate (Fisherbrand P8, Pittsburgh, PA, USA). Ticks were covered with the top case of the Petri plate and sealed with parafilm. Each treatment was repeated 5× (n = 25). Mortality was assessed after 24, 48, and 168 h (= 1 week). Mortality was confirmed if ticks showed no movement after being exhaled upon by the experimenter and if gently poked with fine-tipped forceps.

Acaricidal bioassays were completed on *I. scapularis* to assess the lowest effective concentration to achieve mortality. Two series of solutions, consisting of 1.25, 2.5, 5, 10, and 20% v/v of yarrow HMT and PW flower essential oil were prepared with 1% v/v Tween-80 and deionized water, and mixed for 1 min using a vortexer (Fisher Scientific). A control of just 1% v/v Tween-80 and deionized water was also prepared. Following the acaricidal

bioassays on *I. scapularis*, the series of yarrow HMT flower EO solutions were tested on *D. variabilis* female adults to record any potential acaricidal activity as well.

The main components of yarrow (camphor, *β*-pinene, eucalyptol, and germacrene D) were also tested individually for acaricidal activity. Solutions of each compound at 1.25, 2.5, 5, 10, and 20% v/v were prepared by serial dilutions in 50% v/v ethanol, and 1% v/v Tween-80. Ethanol was used to improve the solubility of some of these compounds in water. Therefore, a second control of 50% v/v of ethanol, 1% v/v Tween-80 and deionized water was prepared and tested on ticks. At each time point (24, 48, and 168 h), $LC_{50}$ values were determined for each compound, HMT flower EO, and PW flower EO.

## Horizontal repellency bioassay

Horizontal repellent bioassays were performed in the Innovation Pavilion Laboratory, Acadia University (Wolfville) using nymphs of *I. scapularis* and *D. variabilis* according to a previously described method (Fig. 1) (Faraone et al. 2019; Wang et al. 2022). Concentric circles of disk filter paper (Fisherbrand P8, 9 cm diameter) of 1.4, 2.5, and 6 cm in diameter were cut, forming four zones: (1) dropping zone, (2) inner circle, (3) treated circle, and (4) outer circle. The ring between the 2.5 and 6.0 cm circles was transferred on a glass Petri plate and treated with 160 µl of the treatment solution under a fume hood. The treated ring was left to dry for about 10 min before to be used in the bioassay. The inner circle (untreated ring) was marked with a circle drawn with a pencil (1 cm diameter) defined as the 'dropping zone'. This area was used to release the ticks at the beginning of the assay. Each filter paper circle (outer circle, treated circle, and inner circle with dropping zone) was reassembled together with forceps in a plastic Petri plate containing a clean filter paper disk covered with a fine layer of unscented petroleum jelly (Vaseline; Unilever Canada, Toronto) used to glue all the section together. The Petri plate was located inside a Plexiglas transparent box (45 × 45 × 45 cm) opened on one side. A humidifier and a thermometer/hygrometer were located inside the transparent box to control environmental parameters during the duration of the experiments. Bioassays were conducted at 20–22 °C and 65–80% RH and were illuminated by fluorescent ceiling lights. Five unfed *I. scapularis* nymphs were transferred using a fine brush into the dropping zone circle. During the experiments,

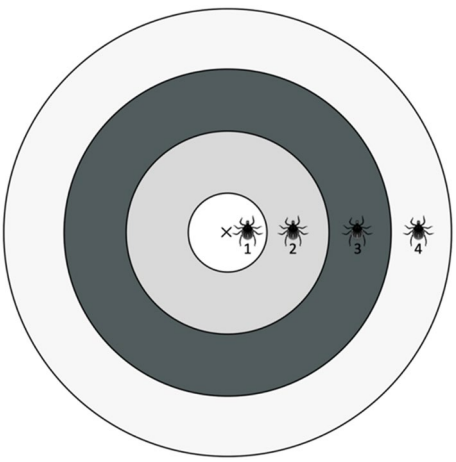


**Fig. 1** Representation of the horizontal bioassay set-up indicating: (1) Dropping zone; (2) Inner circle; (3) Treated circle; (4) Outer circle

an observer was in front of the bioassay arena at 0.3–0.5 m distance. The proximity of the observer stimulated the host-seeking behaviour of ticks enhancing locomotion and activity.

The locations of the ticks were recorded at 5, 30, 60, 90, and 120 min after they were released. At the beginning of the experiment, ticks recorded outside the dropping zone and inside the inner circle were considered as active and included in the data analysis. Those that did not leave the dropping zone during the duration of the experiment were considered not active and excluded from the analysis. Ticks that crossed (crawled) beyond the treated circle and ticks located on the treated circle at the time of position check were recorded as non-repelled, removed from the arena, and discarded. Ticks that entered the treated circle and returned to the inner circle were considered as active and repelled. The observer was always present as a stimulus during the bioassays until the end of the trial. The percentage of ticks repelled was calculated using the formula: % repellency = $[Rt/(NRt + Rt)] \times 100$, where Rt is the number of repelled ticks, and NRt is the number of non-repelled ticks.

Preliminary horizontal bioassays were completed to determine an effective concentration range of yarrow EO for tick repellency. Additionally, preliminary repellency tests were performed using DEET at 25% v/v in hexane to evaluate experimental set-up and tick response (data not published, available upon request). A set of solutions was prepared separately for yarrow HMT and PW flower EO. Concentrations of 2.5, 5, 10, 15, and 20% v/v yarrow flower EO were prepared by serial dilution using hexane as a solvent. Only hexane was used as a control. A full series of treatments (including the control) was completed each day, and the order of tested treatments was done in a randomized fashion. The main components detected in yarrow EOs extracted from flowers and leaves (camphor, $\beta$-pinene, eucalyptol, and germacrene D) were also tested individually for repellency using the same range of concentrations in hexane solution for the determination of $EC_{50}$ values.

### Statistical analysis

Statistical analyses were performed using RStudio (2018). For chemical quantification of the EO, the Grubbs test was used to remove any outlier data points using the 'outliers' package in R (Komsta 2006). For horizontal repellency and acaricidal bioassays (AIT), results were analyzed using the generalized linear mixed-effect regression (glmer) with a binomial link to model the logit of tick repelled. These tests were followed by multiple comparisons of means (Tukey's test), using the 'emmeans' package. For repellency tests, ticks were either treated as repelled (1) or not repelled (0). For AIT, ticks were treated as either dead (1) or alive (0). Acaricidal data were analyzed using a general linear model (lm) using the 'lme4' package. Bioactivity comparison between the two samples of EO was completed using the Kruskal-Wallis test to calculate $\chi^2$ values. Probit analyses were carried out using the MedCalc statistical software (2021) to determine the $LD_{50}$ (acaricidal activity) and $ED_{50}$ (repellency). Values were obtained for both samples of yarrow EO and for the main components of yarrow at various time points. Significance threshold was $\alpha = 0.05$.

## Results

### Essential oil extraction and analysis

Yields of extracted essential oils from various parts of the plant, based on the geographical location of the collection site, are reported in the Supplementary Material

(Table S1). Qualitative and quantitative analyses were performed on the recovered essential oils. Due to a very low amount of EO extracted from the leaves, we were not able to test these oils in tick bioassays. By using standards and comparing the mass spectra from the NIST database library, a total of 21 compounds were identified in the HMT flower EO sample (Table 1). Based on the relative % abundance, main compounds present within the EO from HMT flower sample were camphor (31.1 ± 1.38%), eucalyptol (7.8 ± 0.29%), and germacrene D (9.5 ± 0.51%). A total of 12 compounds were identified in the HMT leaf EO sample through standards and comparison with the NIST database library. Germacrene D (49.1 ± 12.05%) was the most abundant compound present within the EO from HMT. Other minor components were eucalyptol (4.7 ± 1.56%), and iso-borneol (2.5 ± 0.59%). The EO extracted from PW flower presented fewer components (a total of 19) (Table 2). The most abundant compounds were germacrene D (22.6 ± 2.01%), eucalyptol (4.0 ± 0.66%), and β-pinene (2.1 ± 0.01%). Also here, the most abundant peak present within the EO from PW leaves was found to be germacrene D (54.5 ± 0.06%).

**Table 1** Chemical compounds present and mean (±SEM; n = 3) abundance (%) in the essential oils extracted from flowers and leaves of yarrow collected along the Harvest Moon Trail (HMT), Wolfville (Nova Scotia, Canada)

| Chemicals (HMT plants) | Flowers | | Leaves | | ID |
|---|---|---|---|---|---|
| | RT (min) | % Abundance | RT (min) | % Abundance | |
| *α*-Pinene | 8.608 | 0.52 ± 0.06 | | – | S |
| Camphene | 9.09 | 0.61 ± 0.17 | 9.083 | 0.67 ± 0.23 | S |
| *β*-Pinene | 9.907 | 2.25 ± 0.03 | 9.901 | 2.08 ± 0.29 | S |
| *β*-Myrcene | 10.276 | 1.03 ± 0.15 | 10.267 | 1.25 ± 0.23 | S |
| *α*-Phellandrene | 10.717 | 0.44 ± 0.05 | 10.711 | 0.33 ± 0.16 | S |
| 2-Carene | 11.019 | 0.57 ± 0.10 | | – | NIST |
| *p*-Cymene | 11.22 | 1.68 ± 0.12 | 11.213 | 1.11 ± 0.19 | S |
| (*R*)-(+)-Limonene | 11.352 | 0.48 ± 0.13 | | – | S |
| Eucalyptol | 11.42 | 7.82 ± 0.29 | 11.416 | 4.74 ± 1.56 | S |
| γ-Terpinene | 12.109 | 3.77 ± 0.39 | 12.103 | 2.00 ± 0.72 | S |
| Thujone | 13.304 | 1.76 ± 0.11 | 13.304 | 0.30 ± 0.23 | NIST |
| Camphor | 14.259 | 31.05 ± 1.38 | 14.263 | 1.19 ± 0.23 | S |
| Iso-borneol | 14.843 | 8.05 ± 0.37 | 14.841 | 2.52 ± 0.59 | NIST |
| (−)-Terpinen-4-ol | 15.037 | 8.87 ± 0.57 | 15.037 | 1.86 ± 0.30 | S |
| *α*-Terpineol | 15.364 | 2.14 ± 0.94 | | – | S |
| (−)-Bornyl acetate | 17.241 | 4.15 ± 0.34 | 17.238 | 0.81 ± 0.30 | S |
| *β*-Caryophyllene | 19.938 | 3.89 ± 0.93 | | – | S |
| *α*-Humulene | 20.594 | 0.47 ± 0.23 | 19.936 | 2.03 ± 0.49 | S |
| Germacrene D | 21.05 | 9.49 ± 0.51 | 21.048 | 49.07 ± 12.05 | S |
| Naphthalene | 21.672 | 0.84 ± 0.13 | 21.671 | 1.20 ± 0.27 | NIST |
| Chamazulene | 25.197 | 0.67 ± 0.01 | | – | S |
| Unknowns | – | 9.44 ± 2.31 | – | 28.84 ± 15.38 | – |

Chemical identity (ID) is confirmed either by comparison with chemical standards (S) or by the highest National Institute of Standard Technology (NIST) database % match probability

**Table 2** Chemical compounds present and mean (±SEM; n = 3) abundance (%) in the essential oils extracted from flowers and leaves of yarrow collected in Port Williams (PW), Nova Scotia, Canada

| Chemicals (PW plants) | Flowers | | Leaves | | ID |
|---|---|---|---|---|---|
| | RT (min) | % Abundance | RT (min) | % Abundance | |
| $\alpha$-Pinene | 8.606 | 0.28 ± 0.06 | – | – | S |
| Camphene | 9.086 | 0.09 ± 0.09 | – | – | S |
| $\beta$-Pinene | 9.907 | 2.13 ± 0.01 | 9.913 | – | S |
| $\beta$-Myrcene | – | – | 10.282 | – | S |
| p-Cymene | 11.222 | 0.40 ± 0.12 | 11.213 | – | S |
| (*R*)-(+)-Limonene | – | 3.98 ± 0.66 | – | – | S |
| Eucalyptol | 11.420 | 3.98 ± 0.66 | 11.429 | 0.698 ± 0.005 | S |
| $\gamma$-Terpinene | 12.111 | 0.86 ± 0.40 | 12.103 | – | S |
| Camphor | 14.265 | 1.72 ± 0.37 | 14.276 | – | S |
| Iso-borneol | 14.843 | 4.21 ± 0.28 | 14.853 | 0.515 ± 0.07 | NIST |
| (−)-Terpinen-4-ol | 15.037 | 7.65 ± 0.69 | 15.051 | 0.39 ± 0.03 | S |
| $\alpha$-Terpineol | 15.369 | 1.70 ± 0.70 | – | – | S |
| (−)-Bornyl acetate | 17.239 | 0.33 ± 0.09 | 17.244 | – | S |
| Caryophyllene | 19.938 | 4.96 ± 0.35 | 19.936 | 0.975 ± 0.03 | S |
| Humulene | 20.594 | 0.63 ± 0.24 | 20.12 | – | S |
| Germacrene D | 21.047 | 22.57 ± 2.01 | 21.053 | 54.53 ± 0.06 | S |
| $\gamma$-Elemene | | – | 21.314 | 2.63 ± 0.02 | NIST |
| Naphthalene | 21.676 | 3.03 ± 0.23 | 21.676 | 2.27 ± 0.04 | NIST |
| Neroliodol | 22.335 | 8.38 ± 2.01 | – | – | NIST |
| Caryophyllene oxide | 22.813 | 1.08 ± 0.06 | 22.817 | – | NIST |
| $\alpha$-Cadinol | – | – | 23.99 | 1.50 ± 0.03 | NIST |
| Unknowns | 21.672 | 32.01 ± 1.83 | – | 36.50 ± 0.02 | – |

Chemical identity (ID) is confirmed either by comparison with chemical standards (S) or by the highest National Institute of Standard Technology (NIST) database % match probability

## Quantitative analysis

Quantitative analyses were performed using an internal calibration method with eugenol and linalool as internal standards. The main constituents from the HMT and PW flower EO were quantified in ng/mL using linear regression curves (Table 3) and reported in wt% (Table 4). Essential oil from the HMT flowers contained a higher abundance of germacrene D (21.5 ± 1.31 wt %), camphor (9.9 ± 0.08 wt%), and eucalyptol (3.6 ± 0.07 wt%), whereas the EO from PW flowers were richer in germacrene D (26.3 ± 1.57 wt %). EO samples from HMT and PW locations had similar amounts of $\beta$-pinene: 3.8 ± 0.01 wt% and 3.7 ± 0.01 wt%, respectively (Table 4). Compounds present in EO extracted from yarrow leaves were not quantified as those samples were not used in tick bioassays.

## Acaricidal bioassay

Yarrow HMT flower EO exerted significant acaricidal activity on female adult *I. scapularis* (Fig. 2a) with 100% mortality after 24 h for the 20% v/v concentration ($z = -13.137$, $p < 0.001$). After 168 h, 100% ticks were recorded as dead for the 20, 10 ($z = -12.086$,

**Table 3** GC-MS analysis of main compounds present in yarrow flower essential oils from the Harvest Moon Trail, Wolfville, and Port Williams (both NS, Canada), using the selected ion monitoring (SIM) chemical quantification method

| Chemicals | Calibration range (ng/mL) | SIM (m/z ions) | Linear regression curves | Correlation coefficient $R^2$ |
|---|---|---|---|---|
| *β*-Pinene | 5–160 | **93**, 136, 41 | y = 0.0548x – 0.5809 | 0.992 |
| Eucalyptol | 5–160 | **43**, 154, 81 | y = 0.0339x – 0.1851 | 0.993 |
| Camphor | 5–160 | **95**, 152, 41 | y = 0.0288x – 0.2230 | 0.992 |
| Linalool (IS) | 80 | **154**, 93, 71 | – | – |
| Germacrene D | 5–160 | **161**, 204, 91 | y = 0.0066x – 0.0528 | 0.994 |
| Eugenol (IS) | 160 | **164**, 149, 103 | – | – |

Eugenol was used as internal standard (IS) for the quantification of germacrene D, whereas linalool was used as IS for the quantification of *β*-pinene, eucalyptol, and camphor (n = 6). The ions (in bold) are the respective base peak of each compound and were used for quantification, whereas proceeding ions were used as qualifying ions

**Table 4** Chemical quantification (mean ± SEM wt %) of the main components found in yarrow flower essential oils from the Harvest Moon Trail (HMT), Wolfville, and Port Williams (PW) (both NS, Canada)

| Chemicals | HMT | PW |
|---|---|---|
| *β*-Pinene | 3.77 ± 0.01 | 3.71 ± 0.01 |
| Eucalyptol | 3.63 ± 0.07 | 2.67 ± 0.01 |
| Camphor | 9.85 ± 0.08 | 3.01 ± 0.01 |
| Germacrene D | 21.48 ± 1.31 | 25.49 ± 0.76 |

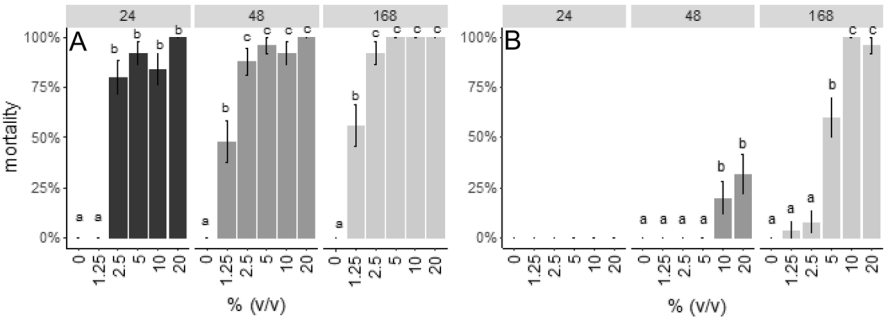


**Fig. 2** Mean (±SEM; n = 25) acaricidal activity of different concentrations (% v/v) of two yarrow flower essential oil (EO) samples from the Harvest Moon Trail (HMT), Wolfville, and Port Williams (PW) (both NS, Canada), at various time intervals (24, 48, and 168 h) against adult female *Ixodes scapularis*: **a** HMT flower EO; **b** PW flower EO. Means within a panel and within a time interval capped with different letters are significantly different (Tukey's test: $p < 0.05$)

$p < 0.001$), and 5% v/v ($z = -12.612$, $p < 0.001$) treatments. Mortality dropped significantly at concentrations of 1.25% v/v ($z = -4.554$, $p < 0.001$) resulting in only 56.0 ± 1.1% mortality after 168 h. Mortality was dose-dependent ($F_{5,442} = 174.582$, $p < 0.001$) with higher concentrations exerting more acaricidal activity. Mortality also increased over time ($F_{2,442}$

= 13.043, $p < 0.001$). In comparison, EO from yarrow PW flowers was less effective on *I. scapularis* (Fig. 2b). After 24 h, no mortality was recorded; however, after 168 h yarrow oil at 10% v/v (z = 8.774, $p < 0.001$) caused 100% mortality.

Comparing the activity of both oils at the same time point, EO from HMT caused significantly higher tick mortality than EO from PW. After 24 h, 20% v/v HMT EO exerted a more effective acaricidal activity compared to the same concentration of PW EO ($\chi^2 = 49$, df = 1, $p < 0.001$). Similarly, 10% v/v HMT EO was stronger than PW EO 10% v/v ($\chi^2 = 35.483$), 5% v/v HMT EO was stronger than PW EO 5% v/v ($\chi^2 = 41.7$), and 2.5% v/v HMT was also stronger than PW 2.5% v/v ($\chi^2 = 32.7$, all df = 1, $p < 0.001$). Only yarrow oil at 1.25% v/v from both collection sites had similar acaricidal activity.

After 48 h, 20% v/v HMT EO exerted a higher acaricidal activity than 20% v/v PW EO ($\chi^2 = 25.2$, df = 1, $p < 0.001$), and a similar trend was reported for lower concentrations: 10% v/v HMT EO was stronger than 10% v/v PW EO ($\chi^2 = 25.8$), 5%v/v HMT EO was stronger than 5% v/v PW EO ($\chi^2 = 45.2$), 2.5% v/v HMT EO was stronger than 2.5% v/v PW EO ($\chi^2 = 38.5$) and 1.25% v/v HMT EO was more acaricidal than 1.25% v/v PW EO ($\chi^2 = 15.5$, all df = 1, $p < 0.001$). Only after 168 h, HMT EO and PW EO had comparable acaricidal activity ($\chi^2 = 1$, df = 1, $p = 0.32$) at the same concentration of 20% v/v.

Among the two oils, yarrow HMT flower EO was selected to be further tested on *D. variabilis* female adult ticks. Although HMT flower EO has shown the most promising acaricidal activity, it exerted minimal acaricidal activity on *D. variabilis* causing only 80% (z = − 6.934, $p < 0.001$) mortality at the highest tested concentration (20% v/v) after 168 h. Overall, EO from yarrow HMT flower did not exert significant acaricidal activity toward *D. variabilis* compared to *I. scapularis*. Mortality was dose-dependent ($F_{5,442} = 23.067$, $p < 0.001$) with higher concentrations having significant acaricidal activity that increased over time ($F_{2,442} = 50.626$, $p < 0.001$) (Figure S1).

$LD_{50}$ values for acaricidal activity were determined for yarrow flower EO and main constituents tested on *I. scapularis* (Table 5); however, mortality data for *D. variabilis* did not fit the Probit model and it was not possible to determine $LD_{50}$ values. After 7 days, the most effective individual compound was germacrene D which exerted the highest acaricidal activity towards *I. scapularis* with an $LD_{50}$ of 2.0% v/v (95% confidence interval = 1.45–2.58). Camphor and *β*-pinene had overlapping $LD_{50}$ values of 3.9% v/v (3.13–4.77) and 5.1 (4.00–6.56), respectively; *β*-pinene and eucalyptol also presented overlapping $LD_{50}$ values of 5.1% v/v (4.00–6.56) and 7.1% v/v (5.76–8.90), respectively. Overall, germacrene D was the most active component, and camphor was better than eucalyptol at killing ticks (Fig. 3). Yarrow HMT EO was more effective than the individual compounds with an $LD_{50}$ of 0.28% v/v (0.69–0.77), indicating that the contribution from the components in the mixture was responsible for the observed effect. EO extracted from HMT yarrow was more effective than EO from PW flower. The latter has an $LD_{50}$ of 4.6%v/v (4.39–4.81) after 7 days, significantly higher than HMT flower EO.

## Horizontal repellency bioassay

Both samples of yarrow flower EO extracted from plants collected in the two locations had a repellent effect against *I. scapularis* nymphs (Table 6). At 30 min, HMT yarrow oil at 15% v/v concentration repelled 88.0 ± 6.6% (z = 13.030, $p < 0.001$) of nymphs. On the other hand, after 30 min, 15% v/v yarrow PW oil repelled 84 ± 7.5% (z = 9.261, $p < 0.001$) of nymphs. The repellent effect decreased over time (HMT, $F_{4,736} = 95.35$; PW, $F_{4,732} = 71.07$), and was dose-dependent (HMT, $F_{5,736} = 46.22$; PW, $F_{5,732} = 71.53$, all $p < 0.001$).

**Table 5** Acaricidal activities ($LD_{50}$, % v/v) of yarrow flower essential oils (EO) and individual main EO components from the Harvest Moon Trail (HMT), Wolfville, and Port Williams (PW) (both Nova Scotia, Canada), at three time intervals

| Treatment | Time (day) | $LD_{50}$ (% v/v) (95% CI) | Slope ± SEM | df | P[#] |
|---|---|---|---|---|---|
| Yarrow HMT flower EO | 1 | 2.41 (1.74–3.35) | − 1.11 ±0.28 | 5 | < 0.001 |
| | 2 | 1.03 (0.39–1.57) | − 0.03 ±0.25 | 5 | < 0.001 |
| | 7 | 0.28 (0.69–0.77) | − 1.92 ± 0.29 | 5 | < 0.001 |
| Yarrow PW flower EO | 1 | * | * | 5 | – |
| | 2 | * | * | 5 | – |
| | 7 | 4.60 (4.39–4.81) | − 5.00 ± 1.50 | 5 | < 0.001 |
| *β*-Pinene | 1 | 13.78 (10.82–19.43) | − 3.34 ± 0.60 | 5 | < 0.001 |
| | 2 | 8.32 (6.54–10.95) | − 2.43 ± 0.38 | 5 | < 0.001 |
| | 7 | 5.12 (4.00–6.56) | − 1.85 ± 0.30 | 5 | < 0.001 |
| Camphor | 1 | 8.29 (6.76–11.28) | − 3.72 ± 0.78 | 4 | < 0.001 |
| | 2 | 6.46 (5.32–8.17) | − 3.27 ± 0.61 | 4 | < 0.001 |
| | 7 | 3.86 (3.13–4.77) | − 2.09 ± 0.37 | 4 | < 0.001 |
| Eucalyptol | 1 | 9.54 (7.86–11.75) | − 3.84 ± 0.62 | 5 | < 0.001 |
| | 2 | 7.72 (6.28–9.57) | − 3.10 ± 0.47 | 5 | < 0.001 |
| | 7 | 7.12 (5.76–8.90) | − 2.79 ± 0.43 | 5 | < 0.001 |
| Germacrene D | 1 | 6.86 (5.99–7.88) | − 7.17 ± 1.36 | 4 | < 0.001 |
| | 2 | 5.77 (4.41–7.55) | − 3.87 ± 0.64 | 4 | < 0.001 |
| | 7 | 2.02 (1.45–2.58) | − 0.86 ± 0.27 | 4 | 0.002 |

Data were determined by performing acaricidal tests on adult female *Ixodes scapularis* adult females (n = 25)

* $LD_{50}$ > 20%v/v; data do not fit the model; [#] Based on probit analysis

By comparing the two different samples, we found notable differences. At specific concentrations and time points, PW flower EO was more effective at repelling ticks compared to HMT flower EO. After 120 min, PW EO at 15% v/v repelled 48 ± 10% of ticks, being more effective than HMT 15% v/v, which repelled only 16 ± 7.5% of ticks ($\chi^2 = 5.76$, df = 1, $p = 0.02$). After 60 min, PW EO 20% v/v repelled 80 ± 8.2% with respect to HMT EO 20% v/v, that repelled only 52 ± 10% of ticks ($\chi^2 = 4.28$, df = 1 $p = 0.04$). Similarly at 60 min, PW EO 5% v/v repelled 24 ± 8.7% compared to HMT EO 5% v/v that repelled none ($\chi^2 = 6.68$, df = 1 $p < 0.001$). At 30 min, PW EO 20% v/v was better than HMT EO at the same concentration ($\chi^2 = 7.98$, df = 1, $p < 0.001$), repelling 100% of ticks compared to HMT EO (72 ± 9.2% of repelled ticks). Finally, after 30 min, PW EO 5% v/v repelled 44 ± 10.0% compared to HMT EO 5% v/v, which was able to repel only 12 ± 6.6% of ticks ($\chi^2 = 6.22$, df = 1, $p = 0.01$).

Yarrow HMT flower EO showed limited to no significant repellent activity against *D. variabilis* nymphs (Figure S2). Yarrow PW flower EO was also tested but did not exert significant repellency against *D. variabilis* nymphs (data not shown).

The effective concentration able to repel 50% ($EC_{50}$) of *I. scapularis* was determined for each yarrow EO sample and for the main constituents of the oil at various time points (Table 7). At the same time point, yarrow EO presented a lower $EC_{50}$ value compared to the individual compounds, indicating the importance of the contribution of the individual components in making the whole mixture more effective at repelling ticks. Yarrow PW

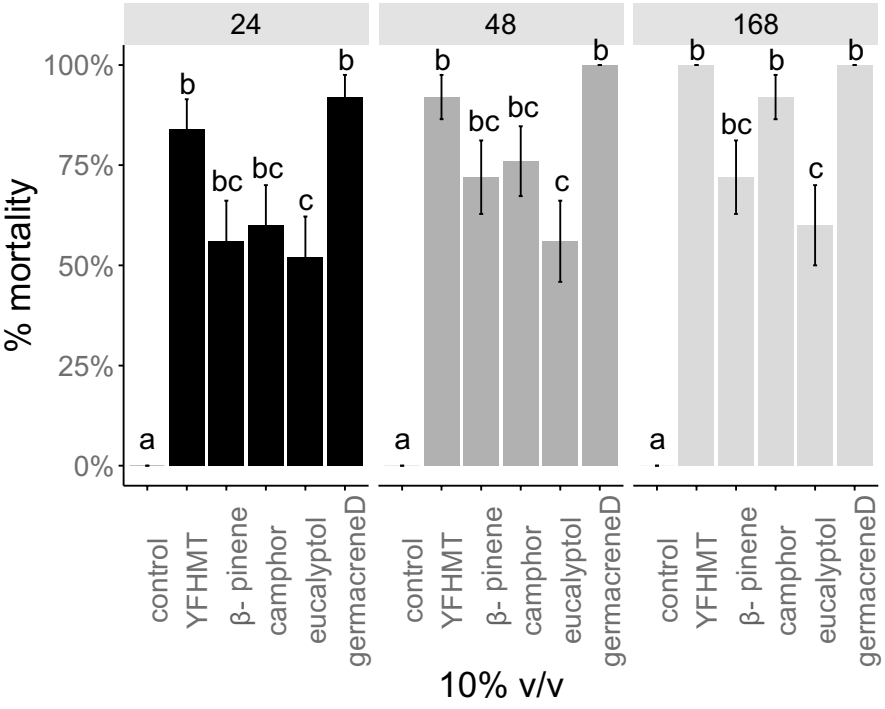


**Fig. 3** Mean (±SEM; n = 25) acaricidal activity of yarrow flower essential oil from the Harvest Moon Trail (Wolfville, NS, Canada) (YFHMT) samples and individual components at the concentration of 10% v/v at various time intervals (24, 48, and 168 h) against adult female *Ixodes scapularis*. Means within a time interval capped with different letters are significantly different (Tukey's test: $p < 0.05$)

**Table 6** Mean (± SEM; n = 25) percentage of *Ixodes scapularis* nymphs repelled by yarrow flower essential oils (EO) from the Harvest Moon Trail (HMT), Wolfville, and Port Williams (PW) (both Nova Scotia, Canada), at different concentrations (0–20% v/v) and time intervals (5–120 min) in horizontal bioassays

| EO source | v/v % | Time interval (min) | | | | | *z* | P[#] |
|---|---|---|---|---|---|---|---|---|
| | | 5 | 30 | 60 | 90 | 120 | | |
| HMT | 0 | 14.3 ±7.8 | 4.0 ±4.0 | 0 | 0 | 0 | – | – |
| | 2.5 | 84.0 ±7.5 | 20.0 ±8.2 | 8.0 ±5.5 | 4.0 ±4.0 | 8.0 ±5.5 | 4.529 | < 0.001 |
| | 5 | 92.0 ±5.5 | 12.0 ±6.6 | 0 | 0 | 0 | 3.625 | < 0.001 |
| | 10 | 80.0 ±8.2 | 52.0 ±10 | 16.0 ±7.5 | 0 | 0 | 5.614 | < 0.001 |
| | 15 | 96.0 ±4.0 | 88.0 ±6.6 | 64.0 ±9.8 | 48.0 ±10 | 16.0 ±7.5 | 13.030 | < 0.001 |
| | 20 | 88.0 ±6.6 | 72.0 ±9.2 | 52.0 ±10 | 28.0 ±9.2 | 20.0 ±8.2 | 10.679 | < 0.001 |
| PW | 0 | 14.3 ±7.8 | 4.0 ±4.0 | 0 | 0 | 0 | – | – |
| | 2.5 | 80.0 ±8.2 | 40.0 ±10 | 16.0 ±5.5 | 0 | 8.0 ±5.5 | 5.450 | < 0.001 |
| | 5 | 96.0 ±4.0 | 44.0 ±10 | 24.0 ±8.7 | 4.0 ±4.0 | 4.0 ±4.0 | 5.899 | < 0.001 |
| | 10 | 100 | 84.0 ±7.5 | 68.0 ±9.5 | 40.0 ±10 | 24.0 ±8.7 | 8.538 | < 0.001 |
| | 15 | 100 | 84.0 ±7.5 | 68.0 ±9.5 | 60.0 ±10 | 48.0 ±10 | 9.261 | < 0.001 |
| | 20 | 100 | 100 | 80.0 ±8.2 | 52.0 ±10 | 36.0 ±9.8 | 9.394 | < 0.001 |

[#] Based on Tukey's test

**Table 7** Repellent activities ($EC_{50}$, % v/v) of yarrow flower essential oils (EO) and individual main EO components from the Harvest Moon Trail (HMT), Wolfville, and Port Williams (PW) (both NS, Canada), at five time intervals (5–120 min)

| Treatment | Time (min) | $EC_{50}$ (% v/v) (95% CI) | Slope ± SE | df | P[#] |
|---|---|---|---|---|---|
| Yarrow HMT flower EO | 5 | 0.05 (0.00–0.36) | 0.70 (±0.19) | 5 | < 0.001 |
| | 30 | 9.20 (6.99–12.22) | − 2.04 (±0.39) | 5 | < 0.001 |
| | 60 | 17.14 (12.12–24.23) | − 3.03 (± 0.55) | 5 | < 0.001 |
| | 90 | * | * | 5 | – |
| | 120 | * | * | 5 | – |
| Yarrow PW flower EO | 5 | 1.43 (0.03–2.20) | − 0.52 (±0.76) | 5 | 0.49 |
| | 30 | 4.11 (2.69–5.41) | − 1.38 (±0.37) | 5 | < 0.001 |
| | 60 | 9.20 (6.97–12.28) | − 2.02 (±0.40) | 5 | < 0.001 |
| | 90 | 15.32 (11.39–25.27) | − 2.28 (±0.44) | 5 | < 0.001 |
| | 120 | * | * | 5 | – |
| Camphor | 5 | 2.63 (1.30–3.76) | − 0.90 (± 0.39) | 5 | < 0.001 |
| | 30 | 10.95 (7.74–15.50) | − 2.41 (± 0.44) | 5 | < 0.001 |
| | 60 | * | * | 5 | < 0.001 |
| | 90 | * | * | 5 | – |
| | 120 | * | * | 5 | – |
| Germacrene D | 5 | 1.58 (0.20–2.93) | − 0.30 (± 0.38) | 4 | 0.43 |
| | 30 | 14.57 (8.81–24.11) | − 1.78 (± 0.41) | 4 | < 0.001 |
| | 60 | * | * | 4 | – |
| | 90 | * | * | 4 | – |
| | 120 | * | * | 4 | – |

Data were determined by performing horizontal repellency bioassays on adult female *Ixodes scapularis* (n = 25)

*$EC_{50}$ > 20% v/v; data do not fit the model

$EC_{50}$ for eucalyptol and *β*-pinene are not reported because data do not fit the model

[#] Based on probit analysis

EO was more effective than the individual compounds with an $EC_{50}$ of 4.1% v/v (95% CI 2.69–5.41) after 30 min, compared to germacrene D and camphor with a higher $EC_{50}$ of 14.6% v/v (8.81–24.11) and 10.9% v/v (7.74–15.50), respectively.

## Discussion

Yarrow essential oils extracted from plants collected in two locations reported quantitative variability in terms of identified chemical metabolites. In addition, they exerted different repellent and acaricidal activities in relation to the tick species tested. Yarrow flower HMT EO was a more effective acaracide, whereas yarrow flower PW EO exerted stronger repellent activity. Differences in bioactivities between these samples are likely the result of the variability in their chemical composition. Yarrow flower HMT EO was richer in camphor and eucalyptol compared to yarrow flower PW EO. The latter was more abundant in *β*-pinene, whereas germacrene D was the main sesquiterpene found in both EO. Yarrow

EO contains mono- and sesquiterpenoids (such as camphor, germacrene D, eucalyptol, and *β*-pinene) with documented pesticidal properties (Bohlmann and Keeling 2008).

Previous studies suggest that camphor and eucalyptol are effective acaricides, and when they are presented in a mixture (such as in an EO) their acaricidal effect is enhanced (Yang et al. 2021). One of the main constituents of balsam fir, *Abies balsamea* (L.) Mill., *β*-pinene has been reported to show significant acaricidal activity on adult *I. scapularis* (Adamo et al. 2022). There are also notable differences in metabolites between the EO extracted from leaves and flowers. In the samples from HMT and PW, EO extracted from the leaves were rich in germacrene D. This sesquiterpene alone has been reported to have significant repellent activity against *Ixodes ricinus* (L.) (Ashitani et al. 2015). Other studies report that EO from *Baccharis dracunculifolia*, a native plant from Brazil and abundant in germacrene D, has substantial acaricidal activity against *Rhipicephalus microplus* (Canestrini) (Zeringota et al. 2015; dos Santos Cardoso et al. 2020). Thus, it would have been beneficial to investigate further the EO from the leaves to assess potential repellent and acaricidal activity, and whether ticks were equally impacted as observed for the EO extracted from the flowers. Unfortunately, not enough EO from the leaves was collected to conduct these tests. Instead, germacrene D was tested alone on *I. scapularis* for repellency and acaricidal activity, along with the other individual main chemical components to determine whether a specific compound was responsible for the observed bioactivities. The results for the AIT showed that germacrene D had the lowest $LD_{50}$ of 2.0% v/v among the four compounds after 7 days, and it is likely to be one of the key compounds responsible for the reported acaricidal activity. However, it was determined that HMT flower EO presented a lower concentration of the compound germacrene D compared to PW flower EO (21.5 vs. 25.5% wt), and yet HMT flower EO had a lower $LD_{50}$ than PW flower EO (0.28 vs. 4.6% v/v). Therefore, other compounds are likely contributing to the significant acaricidal activity exerted by the HMT flower EO sample. The assessment of the acaricidal activity of the individual components revealed that camphor, eucalyptol, *β*-pinene, and germacrene D are all contributing to the toxic effect of the yarrow HMT flowers EO on *I. scapularis* (Fig. 3).

Differences in essential oil composition between samples belonging to the same plant species may be linked to environmental factors such, precipitation, altitude, soil composition and stage of the plant when collected (Yuan et al. 2016). Although yarrow samples studied in this project were collected at comparable sites (i.e., similar longitude, latitude, and altitude) and similar stages of growth (adult plants in full blooming stage at the time of collection), their EO composition was different and presented discrepancies. This might be caused by biotic and abiotic factors; specifically, variables such as soil pH, salinity, texture, and nutrients, may impact EO composition (Karami et al. 2020), and the potential effect of herbivores (Valladares et al. 2002). Additionally, there are many varieties of yarrow found in North America, and the identification between them solely based on morphological features alone is impractical as most of them have similar appearances (Chandler et al. 1982).

Looking at the bioactivities recorded on the two species, *I. scapularis* and *D. variabilis* were impacted differently by the yarrow oil. Yarrow flowers EO had a stronger repellent and acaricidal effect on *I. scapularis* than on *D. variabilis*. *Ixodes scapularis* have a significantly smaller average body size compared to *D. variabilis*. Body size may impact the effectiveness of acaricide action: bigger ticks may be more resistant and likely require a higher concentration to see any detectable effect (Clark 1995). This trend is evident in the AIT results as mortality substantially decreased for *D. variabilis*. Additionally, *D. variabilis* has a hard chitinous outer shell and scutum, that provides excellent protection from its surroundings and shielding penetration of external agents (i.e., essential oils) (Hicks and Elston 2018). Yarrow oils were more effective at repelling *I. scapularis* nymphs than

*D. variabilis* nymphs. It has been reported that ticks present some specificity in response to host odour volatiles (Osterkamp et al. 1999), and this sensitivity might be observed also towards specific EO components reporting variable repellent action (Faraone et al. 2020). In terms of repellency, germacrene D and camphor did not exert a significant repellent action having an $EC_{50}$ of 14.6 and 11.0% v/v after 30 min post-exposure, respectively. On the other hand, yarrow flowers PW EO was a better repellent compared to flowers HMT EO, with an $EC_{50}$ of 9.2% v/v at 1 h post-application. Therefore, the observed effect may be associated to the contribution of the overall essential oil mixture. Unfortunately, the repellent action significantly declined after 1 h for both EO, and this might be due to the high volatility of the oil compounds, causing a reduction of their environmental persistency, and the residual activity at which repellency can be significantly effective (Adamo et al. 2022; Wong et al. 2021).

Our findings have provided more insight on the toxic and repellent properties of common yarrow EO against two tick species of medical and veterinary importance. EO from common yarrow and their main components may have some potential for the management of ticks and might be employed for the development of environmentally friendly strategies for the management of *I. scapularis*.

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**Data availability** Raw data will be made available upon request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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