*Varroa destructor* (Mesostigmata: Varroidae) electrophysiological activity towards common yarrow (Asteraceae) essential oil and its components:   
**supplementary material**

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# Choice behavioral assay

## Method

Choice assays were conducted in an environmentally controlled chamber (Biotronette Mark III, Carolina Biological Supply Company, Burlington, North Carolina, United States of America) using infrared heat lamps (30 ± 2 °C and R.H. 60–70%) and active air ventilation (Thermo Fisher Scientific, Cincinnati, Ohio, United States of America) in a dark room between 18:00 and 21K00 h. Video-recording cameras (Sony Handicam DCR-SR45 and HDR-CX405; Sony of Canada Ltd.; Ontario, Canada) were used to monitor varroa movements for the duration of the assay for later visual analyses.

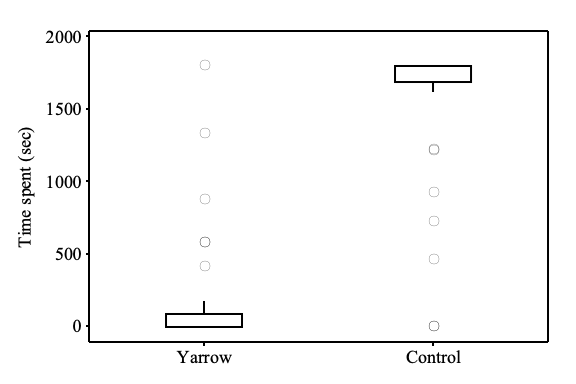
Choice assays were conducted using open-face Petri dishes to avoid oversaturation of volatile components. From 10 to 15 adult female varroa mites were transferred using a moistened paintbrush from a Falcon tube and introduced into the assay arena along the midline, following a method adapted from Peng *et al.* (2015). Plastic Petri dishes (90-mm diameter) were lined with filter paper (85-mm diameter; Fisher Scientific; Ottawa, Ontario, Canada). A pencil was used to draw a midline on the filter paper, and all filter paper discs were subsequently rinsed in 100% laboratory-grade ethanol and air-dried under an active ventilation fume hood for 15 min before use. On the treated side of the filter paper, 50 µL of essential oil (0.1% v/v in hexane following previous research; Light 2019) from yarrow leaves were uniformly applied. On the control side, an equal volume of hexane solvent was applied. Filter paper discs were then air-dried for 15 min under a fume hood.

Mites that did not make a choice or did not move during assays were recorded as unresponsive and excluded from analysis (N = 1). At 30 min, the number of mites located on the yarrow-treated and on the solvent control sides of the filter paper (relative to the midline) was recorded. The time (in seconds) that mites spent on either yarrow-treated or solvent control sides of Petri dish experiments was video recorded for 30 min. A few mites crawled outside of the Petri dish experiments during recordings; these mites were excluded from the above two analyses (N = 2). Choice assays were repeated three times for yarrow treatment *versus* solvent control (N = 34 mites). Individual mites were not considered independent samples, due to possible interaction among mites (Pirk *et al.* 2013). Assays represent a preliminary investigation of possible varroa repellency towards yarrow essential oil, and these were not subjected to further statistical testing.

Some yarrow essential oil components detected in this study are reported elsewhere as arthropod repellents, insecticides and others have specific activities towards *V. destructor* (Table S1). Chemical standards were used to confirm some volatile identities that were detected using gas chromatography – mass spectrometry (Table S2). Complete gas chromatography – mass spectrometry analysis of detectable volatiles from 0.01% v/v yarrow essential oil extract are reported in Table S3.

## Results

Choice assays suggest a strong tendency for varroa mites to choose solvent control sides (32/34; 94%) over 0.1% v/v yarrow-treated sides (2/34; 6%) of Petri dishes at 30 min post-exposure to the essential oil. Video analysis indicated that mites spent more time on solvent control sides than yarrow-treated sides, suggesting the mites avoided the yarrow volatiles (**Fig. S1**).



**Fig. S1.** Time spent by *Varroa destructor* on either the common yarrow-treated (Yarrow) or the hexane solvent control (Control) sides of two-choice Petri dish behavioural assays. Groups of mites were video recorded for 30 min (N = 34 mites). The boxes encompass 50% of data; the whiskers capture remainder of range of data, with open circles that are outliers excepted. The median is the same as the lower bound of the box for Yarrow and the upper bound of the box for Control.

**Table S1.** Individual components of yarrow (*Achillea millefolium*) essential oil with previously described effects on arthropods. Letters refer to previous studies, as listed in the table notes.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Component | Arthropod Repellent | Tick Repellent | Pesticide | Insecticide | Acaricide | \*Varroa destructor |
| (+)--pinene | k |  | k | k |  | b |
| camphene | ﻿a |  | k |  | k |  |
| sabinene |  |  |  | k |  |  |
| -myrcene | k,m |  | k | k |  |  |
| -pinene | k |  | k | k |  | b |
| -﻿phellandrene |  |  | k | g,i |  |  |
| -terpinene | k,o |  | k | r,o | k |  |
| *p*-cymene | k |  | k | k,j |  |  |
| D-limonene | k |  | k | k | k |  |
| eucalyptol  (1,8-cineole) | k | p | k | m | k | b,r |
| -ocimene | d,l |  |  |  |  | n |
| terpinolene | ﻿c,h |  |  | i |  |  |
| linalool | k |  | k | k,m | k | b,e |
| nonanal |  | p |  |  |  |  |
| thujone |  | p | d | d | d | e |
| D-camphor | k,l | q | k |  |  | b,e,r |
| (–)-borneol | r | p |  |  |  | n |
| terpinen-4-ol | c | p | ﻿c | m |  | f |
| -terpineol | k,j | p | k | k,j,m |  | f,t |
| (+ or –)  bornyl acetate | k |  | k | i |  | b |
| eugenol | j | p,s |  | j |  | b |
| -caryophyllene | k |  | k |  |  | e |
| -caryophyllene | c | p |  |  |  |  |
| copaene ( or ) |  | p |  |  |  |  |
| germacrene D |  |  | k |  |  |  |
| (*Z*)-nerolidol |  | p |  |  |  | e |
| carotol | u |  |  |  |  |  |

**\**Varroa destructor*** denotes whether an essential oil component previously demonstrated an undescribed electrophysiological activity from *Varroa destructor* (Von Rudnew and Smeljanez 1969a; Kraus *et al.* 1994b; Ndungu *et al.* 1995c; Lee *et al.* 1997d; Imdorf *et al.* 1999e; Lindberg *et al.* 2000f; Enan 2001g; Yatagai *et al.* 2002h; Park *et al.* 2003i; Enan 2005j; Jaenson *et al.* 2006k; Cook *et al.* 2007l; Ishaaya *et al.* 2007m; Ruffinengo *et al.* 2007n; Siramon *et al.* 2009o; Bissinger and Roe 2010p; Isman *et al.* 2011q; Blenau *et al.* 2012r; Del Fabbro and Nazzi 2013s; Peng *et al.* 2015t; Ali *et al.* 2018u).

**Table S2.** Chemical standards used to confirm identities of some yarrow (*Achillea millefolium*L.) essential oil components through gas chromatography–mass spectrometry.

|  |  |  |
| --- | --- | --- |
| Standard | CAS Number | Vendor |
| benzylaldehyde (> 99%) | 100-52-7 | Sigma-Aldrich |
| -caryophyllene | 87-44-5 | Sigma-Aldrich |
| *p*-cymene | 99-87-6 | Sigma-Aldrich |
| eucalyptol (1,8-cineole) (99%) | 470-82-6 | Fluka |
| linalool (97%) | 78-70-6 | Sigma-Aldrich |
| (*Z*)-nerolidol | 40716-66-3 | Fluka |
| nonyl acetate | 143-13-5 | Sigma-Aldrich |
| (*E*)--ocimene | 13877-91-3 | Sigma-Aldrich |
| -phellandrene | 99-83-2 | Sigma-Aldrich |
| (+)--pinene (98%) | 7785-70-8 | Sigma-Aldrich |
| -terpinene | 99-85-4 | Sigma-Aldrich |
| terpinolene (> 85%) | 586-62-9 | Fluka |
| thujone | 546-80-5 | Sigma-Aldrich |

Sigma-Aldrich, Saint Louis, Missouri, United States of America

Fluka, Mexico City, Mexico

**Table S3.** Volatiles detected from gas chromatography–mass spectrometry of yarrow (*Achillea millefolium*)essential oil at 0.01% v/v in hexane; compiled literature is separated into percent of each volatile identified in leaves, inflorescence, or in both for studies that did not separate plant parts.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| RT | Kovats | CAS | Identity | % Total | Previously Identified% Total Extract | | |
| Leaves a,b | Inflorescence a,b | Both c,d |
| 4.63 | 844 | 6728-26-3; 16635-54-4 | 2-hexenal (*E* or *Z*) | 0.05 |  |  |  |
| 4.68 | 847 | 928-96-1 | 3-hexen-1-ol | 0.02 | 0.6 |  |  |
| 4.95 | 861 | 111-27-3 | 1-hexanol | 0.02 | 0.1 |  |  |
| 5.50 | 886 | 124-11-8 | 1-nonene | 0.18 |  |  |  |
| 5.75 | 901 | 2153-66-4 | santolina triene | 0.11 |  |  |  |
| 6.26 | 921 | 508-32-7 | tricyclene | 0.12 | < 0.01 |  |  |
| 6.34 | 924 | 2867-05-2 | -thujene | 0.24 | < 0.01 |  |  |
| 6.53 | 936 | 80-56-8 | (+)--pinene\* | 2.65 | 0.1–4.5 | 1.8–6.3 | 6.3 |
| 6.94 | 948 | 79-92-5 | camphene | 2.82 | < 0.1-3.7 | < 0.1–2.1 | 2.1 |
| 7.18 | 958 | 100-52-7 | benzaldehyde**\*** | 0.03 |  |  |  |
| 7.54 | 971 | 3387-41-5 | sabinene | 9.44 | 0.1–5 | 2.1–22.3 | 17.6 |
| 7.96 | 988 | 123-35-3 | -myrcene | 2.52 | < 0.1–0.9 | < 0.1–1.1 | 0.8 |
| 8.10 | 993 | 18172-67-3 | -pinene | 0.01 | 7.1–14.1 | 4.6–20.0 | 6.3 |
| 8.43 | 1003 | 99-83-2 | -﻿phellandrene\* | 3.60 |  |  |  |
| 8.73 | 1015 | 99-85-4 | -terpinene\* | 1.24 | < 0.1–1.2 | < 0.1–1.4 | NA |
| 8.93 | 1023 | 52462-29-0 | *p*-cymene\* | 1.37 | < 0.1–4.4 | < 0.1–1.2 | 1.1 |
| 9.08 | 1028 | 5989-27-5 | D-limonene\* | 0.72 |  | 1.0 | 1.3 |
| 9.15 | 1034 | 470-82-6 | 1,8-cineole\* | 5.88 | 0.5–8.5 | 2.7–11.3 | 13.1 |
| 9.41 | 1040 | 122-78-1 | benzene acetaldehyde | 0.02 |  |  |  |
| 9.55 | 1047 | 13877-91-3 | -ocimene\* | 0.12 |  | 0.1 | NA |
| 10.21 | 1068 | 8006-39-1 | -terpineol (*E* or *Z*) | 0.02 |  |  |  |
| 10.49 | 1076 | 21964-44-3 | 1-nonen-3-ol | 0.04 |  |  |  |
| 10.67 | 1087 | 586-62-9 | terpinolene\* | 0.47 | < 0.1 | < 0.1–2.3 | NA |
| 11.05 | 1101 | 78-70-6 | linalool\* | 0.86 |  |  |  |
| 11.20 | 1103 | 124-19-6 | nonanal | 0.01 |  |  |  |
| 11.24 | 1105 | 471-15-8 | -thujone | 0.30 |  |  |  |
| 11.57 | 1116 | 546-80-5 | -thujone\* | 0.06 | 0.4 | < 0.1 | 1.1 |
| 11.80 | 1123 | 29803-82-5 | (*E*)-p-2-menthen-1-ol | 0.07 |  |  |  |
| 12.40 | 1146 | 464-49-3 | D-camphor | 7.53 | < 0.1–6.7 | < 0.1–4.8 | NA |
| 12.90 | 1162 | 67920-63-2 | lilac aldehyde | 0.07 |  |  |  |
| 13.13 | 1166 | 507-70-0 | (–)-borneol | 4.81 | 1.4–8.4 | < 0.1–6.9 | 12.4 |
| 13.37 | 1180 | 562-74-3 | terpinen-4-ol | 3.83 | < 0.1–2.8 | 1.1–2.0 | NA |
| 13.77 | 1194 | 98-55-5 | -terpineol | 1.36 | < 0.1–1.1 | 0.4–1.5 | 1.1 |
| 14.16 | 1211 | 240-777-5 | (*E*)-piperitol | 0.05 | < 0.1 | < 0.1 |  |
| 15.07 | 1238 | 122-03-2 | cuminal | 0.08 |  |  | NA |
| 16.25 | 1283 | 76-49-3 | (+ or –) bornyl acetate | 2.69 | 0.1–6.0 | 0.3–3.6 | 8.1 |
| 16.75 | 1302 |  |  | 0.05 |  |  |  |
| 17.56 | 1333 | 515-00-4 | myrtenol | 0.02 |  |  | 0.1 |
| 18.02 | 1350 | 97-53-0 | eugenol | 0.09 |  |  | NA |
| 18.55 | 1370 | 14912-44-8 | -ylangene | 0.09 |  |  |  |
| 18.72 | 1376 | 17699-14-8 | -cubebene | 0.07 | < 0.1–0.9 | < 0.1–0.4 |  |
| 18.93 | 1385 | 5208-58-2; 5208-59-3 | bourbonene ( or) | 0.03 |  |  |  |
| 19.10 | 1390 | 33880-83-0 | (+ or –) -elemene | 2.18 |  |  |  |
| 19.28 | 1398 | 644-30-4 | -curcumene | 0.02 | < 0.1–1.6 | < 0.1–1.8 |  |
| 19.85 | 1419 | 87-44-5 | -caryophyllene\* | 3.49 | 0.8–5.1 | 2.2–7.7 | 2.3 |
| 19.95 | 1425 | 451-56-9 | -curcumene | 0.11 |  |  |  |
| 20.46 | 1445 | 24268-39-1 | -muurolene | 0.04 |  |  |  |
| 20.62 | 1450 | 3853-83-6 | -himachalene | 0.29 |  |  |  |
| 20.74 | 1456 | 6753-98-6 | -caryophyllene | 0.55 | < 0.1 | < 0.1–0.7 | NA |
| 20.86 | 1461 | 3856-25-5; 18252-44-3 | copaene ( or ) | 0.01 |  |  | 0.1 |
| 21.10 | 1463 | 3691-12-1; 88-84-6 | guaiene ( or ) | 0.04 |  |  |  |
| 21.20 | 1474 | 18431-82-8 | -chamigrene | 0.36 |  |  |  |
| 21.26 | 1477 | 25246-27-9 | alloaromadendrene | 0.08 |  |  |  |
| 21.40 | 1482 | 37839-63-7 | germacrene D | 2.97 | < 0.1–49.2 |  | 1.5 |
| 21.42 | 1483 | 118-65-0 | isocaryophyllene | 0.58 |  |  |  |
| 21.60 | 1491 | 28624-23-9 | -selinene | 0.96 |  |  |  |
| 21.68 | 1494 | 495-60-3 | zingiberene | 0.01 |  |  |  |
| 21.73 | 1496 | 22567-17-5 | -gurjunene | 0.30 |  |  |  |
| 21.80 | 1500 | 10208-80-7 | -muurolene | 0.20 | < 0.1–0.7 | < 0.1–0.6 |  |
| 21.84 | 1501 | 1461-03-6 | -himachalene | 0.25 | < 0.1–0.7 | < 0.1–0.7 |  |
| 21.89 | 1503 | 502-61-4 | -farnesene | 0.12 | 0.7 |  | NA |
| 22.00 | 1508 | 473-13-2; 17066-67-0 | selinene ( or ) | 0.11 |  |  |  |
| 22.14 | 1514 | 483-74-9 | -cadinene | 0.13 |  |  |  |
| 22.26 | 1519 | 483-76-1 | -cadinene | 0.58 | < 0.1–1.2 | < 0.1–7.6 | 0.9 |
| 22.37 | 1523 | 20307-83-9 | -sesquiphellandrene | 0.03 |  |  |  |
| 22.95 | 1548 | 639-99-6; 32142-08-8 | elemol ( or ) | 0.36 |  |  |  |
| 23.23 | 1557 | 40716-66-3 | (*Z*)-nerolidol\* | 0.44 | 1–14.1 | 11.6–31.9 |  |
| 23.41 | 1567 | 13567-39-0 | -cedrene epoxide | 0.05 |  |  |  |
| 23.64 | 1577 | 6750-60-3 | (*E*)-spathulenol | 0.04 | 1.3–10.2 | < 0.1–2.4 |  |
| 23.77 | 1582 | 1139-30-6 | caryophyllene oxide | 0.52 | 0.1–23.0 | 0.1–4.6 |  |
| 23.86 | 1586 | 72747-25-2 | isoaromadendrene epoxide | 0.01 |  |  |  |
| 24.05 | 1594 | 88034-74-6 | (*Z*)--bergamotol | 0.17 |  |  |  |
| 24.09 | 1596 |  |  | 0.05 |  |  |  |
| 24.40 | 1607 | 465-28-1 | carotol | 0.05 |  |  |  |
| 24.50 | 1613 | 473-15-4 | -eudesmol | 0.02 |  |  |  |
| 24.73 | 1626 |  |  | 0.16 |  |  |  |
| 24.85 | 1630 | 28296-85-7 | -acorenol | 0.18 |  |  |  |
| 24.91 | 1633 | 15051-81-7 | -eudesmol | 0.41 | < 0.1–12.2 | < 0.1–3.3 |  |
| 25.13 | 1642 | 5937-11-1 | -epi-cadinol | 0.19 | < 0.1 | 1.1 | 0.9 |
| 25.20 | 1644 | 19912-62-0 | -epi-muurolol | 0.19 |  |  |  |
| 25.22 | 1646 | 19435-97-3 | (–)--cadinol | 0.09 |  |  |  |
| 25.30 | 1650 | 41370-56-3 | -copaen-11-ol | 0.46 |  |  |  |
| 25.42 | 1655 | 19912-67-5 | cubenol | 1.71 |  |  |  |
| 25.50 | 1658 | 577-27-5 | ledol | 0.33 |  |  |  |
| 25.70 | 1667 | 18319-40-9 | 8-cedren-13-ol | 0.32 |  |  |  |
| 26.10 | 1684 | 145512-84-1; 58319-05-4 | sesquisabinene hydrate (*E* or *Z*) | 0.53 | < 0.1–1.6 | < 0.1–1.2 |  |
| 27.10 | 1730 | 529-05-5 | chamazulene | 5.48 | < 0.1–1.4 | < 0.1–2.7 | 5.3 |
| 27.20 | 1735 |  |  | 0.01 |  |  |  |
| 35.23 | 2500 | 629-99-2 | pentacosane | 0.05 |  |  | < 0.1 |
| 36.49 | 2600 | 630-01-3 | hexacosane | 0.03 |  |  | < 0.1 |
|  |  |  | others\*\* | 21.01 |  |  |  |

Compounds marked \* were confirmed using chemical standards (see Table S2)

**RT** = retention time using DB-5 capillary column

**Kovats** = retention index determined from hydrocarbon standard series (C8-C20)

**CAS** = Chemical Abstracts Service registry number

**Identity** = compound identity based on the National Institute of Standards and Technology (NIST) database match, Kovats index match, and supporting literature; all identities had NIST reverse match between 700 and 900 and matched retention times or indices reported in published literature

**% total** = percent of total compounds detected, with total amount of all essential oil components being 109.6 ng/µL of 0.01% essential oil determined using nonyl acetate internal standard at 3 ng/µL

**Previously Identified Percent** = compounds reported by other literature and their range in percent abundance when available for yarrow **Leaves** and **Inflorescence** (aJaenson *et al.* 2006; bJudzentiene and Mockute 2010) and entire plant (**Both**) (cChandler *et al.* 1982; dNadim *et al.* 2011), **NA** indicate chemical detection with no percent abundance given

**others**\*\* = all other unidentified essential oil components, excluding those that elicited electrotarsal responses from *Varroa destructor*.