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ORIGINAL PAPER

Eco-friendly control of the poultry red mite, *Dermanyssus gallinae* (Dermanyssidae), using the α-thujone-rich essential oil of *Artemisia sieberi* (Asteraceae): toxic and repellent potential

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Abstract The poultry red mite, *Dermanyssus gallinae*, represents a key threat for the poultry industry worldwide. The control of *D. gallinae* is mainly achieved by continuous applications of acaricides. However, the fast-growing development of resistance, and the strict laws concerning chemicals admitted for treatments on food animals, highlighted the importance of alternative control tools. Here, we explored the potential of *Artemisia sieberi* essential oil against *D. gallinae*. In this study, the *A. sieberi* essential oil was analyzed using GC and GC-MS. The oil toxicity through contact and fumigant assays on adult mites was evaluated. The oil repellent activity was assessed on adult mites over different time intervals. Lastly, the residual toxicity of various doses of the oil was evaluated on *D. gallinae* until 14 days post treatment. GC and GC-MS showed that the oil was rich in α-thujone (31.5%), β-thujone (11.92%), camphor (12.3%), and 1,8-cineole (10.09%). Contact toxicity on adult mites showed 50% lethal concentration (LC_{50}), LC_{90}, and LC_{99} of 15.85, 26.63, and 35.42 $\mu g/cm^3$, respectively. In fumigant assays, the oil was toxic on *D. gallinae*, and mortality was significantly higher in open containers over closed ones, underlining the key role of highly volatile constituents. Repellent assays showed that after 24 h from the treatment, all doses of the *A. sieberi* essential oil led to significant repellent activity over the control, except for 2 $\mu g/cm^3$. After 48 h, *A. sieberi* essential oil tested at all doses led to significant repellent activity, if compared to the control. Residual toxicity assays showed that time exposure and concentration tested had a significant impact on mite mortality after 1, 2, 5, and 7 days from the treatment. Notably, mortality remained significantly higher over the control for 7 days after spraying with oil at 2%. Further field assays with selected molecules from the *A. sieberi* essential oil are ongoing, testing them in synergistic blends, as well as in microencapsulated formulations.

Keywords Biopesticide · Contact toxicity · Fumigant assay · Mite control · Poultry red mite · Residual toxicity

Introduction

Dermanyssus gallinae (Acari, Dermanyssidae) (Fig. 1a), also known as the poultry red mite, represent a key threat for the poultry industry, with special reference to laying hen, in many parts of the world, including the USA, Europe, Japan, China, and Iran (Chauve 1998; Sparagano et al. 2009; Wang et al. 2010; Barimani et al. 2016). Studies conducted in Iran revealed that *D. gallinae* is the most prevalent blood-feeding mite in the breeder and caged layer flocks (Rahbari et al. 2009). The blood-feeding behavior of *D. gallinae* leads to stress, restlessness, irritation, anemia, and even death in heavy infestation cases, due to exsanguinations (Kirkwood 1968). Furthermore, poultry red mites are potential vectors of several pathogens, including *Salmonella enterica* (Hamidi et al. 2011), *Erysipelothrix rhusiopathiae* (Chirico et al. 2003), and *Avipoxvirus* (Chikuba et al. 2008). From an economical point of view, *D. gallinae* causes high production losses,

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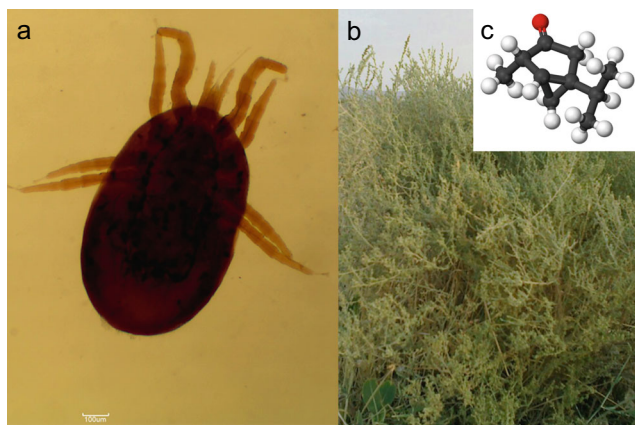
Fig. 1 **a** The poultry red mite, *Dermanyssus gallinae* (Acari: Dermanyssidae). **b** Plants of *Artemisia sieberi* (Asteraceae). In this study, a thujone-rich essential oil (**c**) of *A. sieberi* was tested for its toxic and repellent activity on *D. gallinae*

including decreased meat (15%) and egg production (15–20%), and may even cause death of its hosts in a reduced amount of cases (6–7%) (Kilpinen et al. 2005). Particularly, the production in laying hens is affected through a decline in the growth rate and great decreases in egg production and egg quality (i.e., shell thinning and blood spotting on the shell surface) (Chauve 1998).

The control of poultry red mite populations is primarily achieved by continuous applications of acaricides, including organophosphates, pyrethroids, and carbamates. The fast-growing development of resistance to pesticides currently marketed (Naqqash et al. 2016), as well as the strict laws concerning the chemicals admitted for treatments on food animals, underlined the key importance to develop alternative methods for the control of *D. gallinae* (Kim et al. 2004, 2007; Nechita et al. 2015; Tabari et al. 2015). One alternative tool can be the formulation of novel acaricides using plant-derived compounds, including essential oils. However, botanical pesticide research has been mostly focused on mosquito (Benelli 2015; Pavela and Benelli 2016a, b) and tick control (Pavela et al. 2016), while studies on other arthropod pests and vectors of medical and veterinary importance still remain scarce (Benelli et al. 2017a). To the best of our knowledge, only few researches have focused on the potential employ of essential oil-based pesticides or repellents against the poultry red mite *D. gallinae* (Kim et al. 2004, 2007; George et al. 2009, 2010; Tabari et al. 2015; Masoumi et al. 2016).

The genus *Artemisia* represents a source of molecules and herbal preparations of high parasitological interest, as recently highlighted by the Nobel Prize to Y. Tu for the discovery of artemisinin (Benelli and Mehlhorn 2016). Besides antiplasmodial drugs extracted from *Artemisia vulgaris*, other *Artemisia* species, such as *Artemisia absinthium*, have been recently reported for their promising toxicity against arthropod vectors, with special reference to mosquitoes (Govindarajan and Benelli 2016). *Artemisia sieberi* Besser

(Asteraceae) (Fig. 1b) is a dry land plant, mainly growing in Southwest and Central Asia. It is widely distributed in the semi-desert and desert areas of Iran (Mahboubi and Farzin 2009). This aromatic plant species has been studied for antimicrobial, antifungal, and anticoccidial activity (Arab et al. 2006; Khosravi et al. 2003; Mahboubi and Farzin 2009). In addition, it has been reported that the essential oil of *A. sieberi* showed a fumigant toxicity against several stored product pests of economical importance (Negahban et al. 2007). On this basis, here, we decided to explore the potential of *A. sieberi* essential oil against the poultry red mite, *D. gallinae*. In this study, the *A. sieberi* essential oil was analyzed using gas chromatography (GC) and GC-MS analyses. Furthermore, we evaluated the essential oil toxicity through contact and fumigant assays on adult mites. The repellent activity of *A. sieberi* essential oil on *D. gallinae* was assessed over different time intervals. Lastly, the residual toxicity of various doses of the oil was evaluated until 14 days post treatment.

Material and methods

Essential oil extraction and GC and GC-MS analyses

The aerial parts of *A. sieberi* at full-flowering stage were collected in Arak (34° 06′ 33.1″ N, 49° 47′ 35.5″ E) (Markazi Province, Iran) during September 2014. Voucher specimens of the plant were identified at species level at the Arak Agricultural Sciences University (Arak, Iran). Plant aerial parts were shade-dried at 25 °C and then hydro-distilled by using a Clevenger-type apparatus to extract the essential oil. Essential oil extraction was done according to Negahban et al. (2007). GC analysis was performed using a Shimadzu GC-9A with helium as a carrier gas on a DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 mm). GC-MS was carried out on a Varian 3400 GC-MS system equipped with a DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 mm), and oven temperature was 40–250 °C at a rate of 4 °C, with the following conditions: transfer line temperature, 260 °C; carrier gas, helium with a linear velocity of 31.5 cm/s; split ratio, 1:60; ionization energy, 70 eV; scan time, 1 s; and mass range, 40–300 amu. The identification of the *A. sieberi* essential oil compounds was based on the comparison of their retention indices and mass spectra with those in commercial libraries NIST 98.1 and MassFinder 3.1. The concentration of each essential oil component was calculated from the integration area of the chromatographer (Govindarajan and Benelli 2016).

Mites

D. gallinae adults used in experiments were collected from a commercial laying poultry farm in Gorgan (Iran). Mites were

stored in a sealable, transparent glass container and stored at 25 ± 1 °C and $55 \pm 5\%$ relative humidity (R.H.) for 24 h before the testing phase.

Contact toxicity

Contact toxicity assay was done according to the method described by Tabari et al. (2015). Briefly, we prepared different dilutions of the *A. sieberi* essential oil in 50 μl ethanol, leading to 2, 5, 10, 20, and 40 μg/cm³. The *A. sieberi* essential oil was applied on Whatman No. 1 filter papers. After drying, treated filter papers were placed on the bottom of Petri dishes (4.8 cm diameter × 1.4 cm). Then, 150 adult mites were introduced into each Petri dish containing treated filter papers and then sealed with Parafilm. Control filter paper discs were treated using only 50 μl of ethanol. All assays were done in the same condition at 25 ± 1 °C and $55 \pm 5\%$ R.H. Three replicates were carried out concurrently for all tested groups of mites.

Fumigant toxicity

To determine whether the lethal activity of the essential oil on *D. gallinae* was related to contact or fumigant toxicity, the experimental apparatus described by Tabari et al. (2015) was used. It avoided direct contact of mites with filter papers. Vessels with mites in them were either sealed with a layer of Parafilm (*method A*, hereafter) or left uncovered (*method B*). The *D. gallinae* mortality rates were determined after 24 h under a loop by prodding mites with a pin. If no movement was observed, mites were considered as dead.

Repellent activity

The method used to assess the repellent activity of *A. sieberi* essential oil was described by Masoumi et al. (2016). Four conical flasks and a Y-tube olfactometer were assembled to develop a repellence measuring system. The air was pumped into the system at a rate of 1 l/min and filtered by passing through activated carbon; bubbling into distilled water humidified it. Then, using a Y-tube olfactometer, airflow was divided equally and each airflow was conducted to a 250-ml Erlenmeyer flask. One of these flasks contained the *A. sieberi* essential oil formulated on filter paper, while the other one contained the distilled water on filter paper. Air from these flasks was then directed to an olfactometer, where *D. gallinae* mites were released by using 0.2-mm mesh overall openings. In each assay, 50 *D. gallinae* adults were placed into the Y-tube olfactometer. The distribution of mites in the two opposing arms of the Y-tube olfactometer was recorded after 30 min. Then, the filter papers treated with four different concentrations of *A. sieberi* essential oil were added to the system and the number of mites in the untreated arm was recorded after 24 and 48 h. Three replications were used for each tested

combination. Between successive runs of the experiment, all glassware exposed to test botanical products were washed with ethanol and finally rinsed in double-distilled water.

Residual toxicity assay

Three different concentrations of the *A. sieberi* essential oil (i.e., 0.5, 1, and 2%) evaluated over five different exposure times were tested in residual toxicity assay. The solutions containing *A. sieberi* essential oil were sprayed on aluminum foil surfaces, and then *D. gallinae* mites were exposed to the surfaces at 1, 2, 5, 7, and 14 days post spraying. The area of exposure on previously sprayed surfaces was limited using a converted Petri dish. Vaseline was applied to edges of Petri dishes to ensure mite's contact on treated surfaces. For each solution and time exposure, three replicates were performed.

Table 1 Chemical composition of *Artemisia sieberi* essential oil

Compound	Retention index	Peak area (%)	Mode of identification
α-Thujene	922	0.6	RI, MS
α-Pinene	932	1.22	RI, MS
Camphene	945	8.72	RI, MS
Sabinene	969	0.3	RI, MS
β-Pinene	975	0.98	RI, MS
Myrcene	986	0.3	RI, MS
α-Terpinene	1012	0.26	RI, MS
ρ-Cymene	1019	1.03	RI, MS
1,8-Cineole	*1028*	*10.09*	RI, MS
γ-Terpinene	1052	0.62	RI, MS
Linalool	1070	0.64	RI, MS
Artemisia alcohol	1080	0.23	RI, MS
α-Thujone	*1102*	*31.5*	RI, MS
β-Thujone	*1112*	*11.92*	RI, MS
Myrcenol	1123	0.37	RI, MS
Camphor	*1140*	*12.3*	RI, MS
cis-Verbenol	1143	0.35	RI, MS
Pinocarvone	1149	1.22	RI, MS
trans-Verbenol	1160	0.89	RI, MS
Borneol	1166	1.2	RI, MS
p-Cymen-8-ol	1176	1.14	RI, MS
Myrtenol	1192	0.3	RI, MS
cis-Piperitol	1196	0.26	RI, MS
trans-Piperitol	1206	0.54	RI, MS
Piperitone	1224	1.8	RI, MS
Thymol	1248	0.3	RI, MS
Total	–	89.08	–

The molecules given with values in italic represent the main essential oil constituents

RI retention index, *MS* mass spectra

Table 2 Contact toxicity of the α-thujone-rich *Artemisia sieberi* essential oil against *Dermanyssus gallinae* adults

Dose (μg/cm^3)	Mortality (%) $\pm$ SE	LC$_{50}$ (μg/ml) (95% LCL–UCL)	LC$_{90}$ (μg/ml) (95% LCL–UCL)	LC$_{99}$ (μg/ml) (95% LCL–UCL)	χ^2 (d.f.)
2	3.55 $\pm$ 0.22[a]	15.85 (15.17–16.61)	26.63 (25.27–28.7)	35.42 (33.9–37.8)	1.82 (5), n.s.
5	10.88 $\pm$ 0.22[b]				
10	25.77 $\pm$ 0.44[c]				
20	68.44 $\pm$ 0.23[d]				
40	100 $\pm$ 0.00[e]				

Within the column, different letters indicate significant differences among treatments (ANOVA, Tukey's HSD, $P < 0.05$)

LCL lower confidence limit, *UCL* upper confidence limit

All assays were done in the same condition at 25 $\pm$ 1 °C and 55 $\pm$ 5% humidity. Mite mortality (%) was determined after 48 h from the end of the exposure to the *A. sieberi* essential oil treatment.

Statistical analysis

To determine LD$_{50}$, LD$_{90}$, and LD$_{99}$ values, mite mortality data were subjected to Probit analysis, and chi squares were not significant (Benelli 2017). Data of the fumigant toxicity, repellent activity, and residual toxicity assays were analyzed using ANOVA followed by Tukey's HSD test. Values of $P < 0.05$ were considered significant. All the statistical analyses were carried out using SPSS, version 16.

## Results and discussion

GC and GC-MS analyses

Our GC and GC-MS analyses showed that the major constituents of the *A. sieberi* essential oil were α-thujone (31.5%) (Fig. 1c), β-thujone (11.92%), camphor (12.3%), and 1,8-cineole (10.09%), as shown in Table 1. To the best of our knowledge, previous investigations shedding light on the chemical composition of *A. sieberi* essential oil showed several key quantitative and qualitative differences. For example, Weyerstahl et al. (1993) noted that major constituents of the *A. sieberi* essential oil are camphor (44%), 1,8-cineole (19%), camphene (5%), terpinen-4-ol (2.5%), and α-terpineol (2%). In addition, the main sesquiterpene component is dehydro-1,8-sesquicineole. Sefidkon et al. (2002) highlighted the high oil yield of *A. sieberi* (1.02%), reporting camphor (49.3%), 1,8-cineole (11.1%), and bornyl acetate (5.8%) as main molecules. Later on, Negahban et al. (2007) reported that the *A. sieberi* essential oil mainly contained camphor (54.7%), camphene (11.7%), 1,8-cineol (9.9%), β-thujone (5.6%), and α-pinene (2.5%). We hypothesize that these differences can be due to the different collection sites [i.e., Qom Province, Iran (Negahban et al. 2007); Arak, Markazi Province, Iran (our study)] and harvesting periods; indeed, we collected flowering aerial parts, while plant material at vegetative stage was probably collected by Negahban et al. (2007).

Toxic and repellent activity

Pest management strategies relying to conventional pesticides have been progressively hindered by the fast-growing development of arthropod resistance (Naqqash et al. 2016), increases in consumers demand for safe and residue-free foodstuffs, and a decrease in available products due to stricter legislation (George et al. 2009; Pavela and Benelli 2016b). This has lead to considerable market opportunities for alternative products (Khater et al. 2016), including botanical pesticides (Benelli 2015). Selected plant extracts, isolated metabolites, and essential oils meet the criteria of minimum risk pesticides, so many researches recently focused on them as potential starting materials to develop eco-friendly pesticides (Kim et al. 2004; Abdel-Ghaffar et al. 2008, 2009; Semmler et al. 2009; Schmahl et al. 2010; Benelli and Mehlhorn 2016).

Notably, research on eco-friendly management of vectors and parasites showed that a wide number of botanicals have promising toxic and repellent properties, also on several mite and tick species of high economical importance (Mehlhorn et al. 2005; Govindarajan et al. 2016a, b, c; Benelli et al. 2016; Pavela
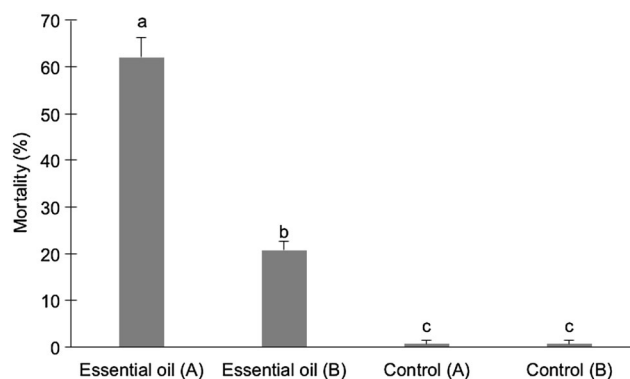
Fig. 2 Fumigant toxicity of α-thujone-rich *Artemisia sieberi* essential oil against the poultry red mite, *Dermanyssus gallinae*: closed (*A*) and open (*B*) arena assays showing the LD$_{50}$ value estimated on adult mites. *T-bars* represent standard errors. *Above each column, different letters* indicate significant differences among treatments (ANOVA, Tukey's HSD, $P < 0.05$)

Table 3 Repellence of the α-thujone-rich *Artemisia sieberi* essential oil against *Dermanyssus gallinae* over two exposure times

Time elapsed	Repelled mites (n)				Control
	2 µg/cm³	5 µg/cm³	10 µg/cm³	20 µg/cm³	
Before treatment	19.33 ± 1.76[a]	20.66 ± 4.37[a]	20 ± 2[a]	20 ± 2.51[a]	18.66 ± 0.88[a]
24 h post treatment	25.66 ± 0.66[a]	28 ± 1.15[a]	32 ± 1.52[b]	33 ± 1.1[b]	20.66 ± 1.76[a]
48 h post treatment	28 ± 0.57[b]	34 ± 0.57[c]	37 ± 1.15[c]	41.66 ± 1.2[c]	19.33 ± 2.33[a]

Values are means followed by standard errors. Within each column, different letters indicate significant differences among treatments (ANOVA, Tukey's HSD, $P < 0.05$)

et al. 2016, Mehlhorn 2016). However, limited information is still available about the eco-friendly control of poultry red mites using botanical pesticides. Locher et al. (2010) described a neem-based product, Mite-Stop®, as an effective botanical acaricide for the control of the poultry red mite, while George et al. (2009) showed that essential oils of thyme, manuka, and pennyroyal tested on *D. gallinae* achieved 99% lethal concentration (LC_{99}) values lower than 0.30 mg/cm³. Furthermore, based on a comparison of LD_{50} values, Kim et al. (2004) have reported that acaricidal activity of some plant extract preparations on *D. gallinae* adults was almost comparable to that of the synthetic pesticide profenofos. These authors screened 56 essential oils using both filter paper assays and fumigation toxicity tests. In contact bioassays, 100% mortality testing 0.07 mg/cm² has been reported for bay, cade, cinnamon, clove bud, coriander, horseradish, lime, mustard, pennyroyal, pimento berry, spearmint, red thyme, and white thyme oils (Kim et al. 2004).

Mortality rates achieved testing the α-thujone-rich *A. sieberi* essential oil on *D. gallinae* in contact toxicity assays are presented in Table 2. The essential oil of *A. sieberi* showed significant toxicity against the poultry red mite with LC_{50}, LC_{90}, and LC_{99} values of 15.85, 26.63, and 35.42 µg/cm³, respectively. The toxic effects of *A. sieberi* essential oil in fumigant assays on *D. gallinae* are given in Fig. 2. The comparison of mortality rates in *A. sieberi* essential oil-treated groups and controls showed that *A. sieberi* essential oil tested using closed and open containers led to significant mortality in *D. gallinae*; mortality rate using the closed container method (A) was significantly higher over the open container method (B) ($P < 0.05$). In fumigant assays, the highest mortality rate was observed for the *A. sieberi* essential oil tested with the

closed container method, which was statistically significant in comparison to all the other groups ($P < 0.05$). In agreement with our findings, fumigation tests carried out by Kim et al. (2004) at 0.28 mg/cm² on poultry red mites showed that cade, clove bud, coriander, horseradish, and mustard oils were effective in closed containers than in open ones, pointing out that the effect of these essential oils can be largely due to the action in the vapor phase (Kim et al. 2004).

Furthermore, in repellent activity assays, before initiation of treatments, no significant differences were detected about the distribution of *D. gallinae* in the arms of the olfactometer ($P > 0.05$) (Table 3). After 24 h, all doses of the α-thujone-rich *A. sieberi* essential oil led to significant repellent activity in comparison to the control ($P < 0.05$), except for the dose of 2 µg/cm³. After 48 h, *A. sieberi* essential oil tested at all doses led to significant repellent activity, if compared to the control ($P < 0.05$). As it could be expected, the highest repellency was seen in the highest dose of essential oil after 48 h.

Data obtained from residual toxicity assays showed that time exposure and concentration tested had a significant impact on poultry red mite mortality at time intervals of 1, 2, 5, and 7 days post application ($P < 0.05$) (Table 4). Mean mortality rates of mites remained significantly higher over those of the control for 7 days after spraying for 2% concentration ($P < 0.01$). For 0.5% α-thujone-rich *A. sieberi* essential oil, significant mortality in comparison to the control was maintained for 5 days ($P < 0.001$). When testing 1% concentration, this time was significantly longer, reaching to 7 days ($P < 0.05$). Overall, several essential oils and plant-derived products have been tested for their acaricidal activity on *D. gallinae*. However, it has been also pointed out that the

Table 4 Residual toxicity of different concentrations of the α-thujone-rich *Artemisia sieberi* essential oil tested on *Dermanyssus gallinae* mites

Treatment	Time post treatment (days)				
	1	2	5	7	14
0.5% essential oil	26.44 ± 1.93[a]	14.11 ± 1.41[a]	7.77 ± 0.8[a]	1.33 ± 0.76[b]	0.22 ± 0.22[b]
1% essential oil	55.33 ± 1.01[c]	22.44 ± 2.47[c]	8.44 ± 0.89[a]	2.44 ± 0.22[a]	0.44 ± 0.22[b]
2% essential oil	75.99 ± 4.43[d]	35.99 ± 2.14[d]	10.66 ± 0.38[a]	3.1 ± 0.22[a]	0.88 ± 0.22[b]
Control	0.00[b]	0.66 ± 0.387[b]	0.44 ± 0.44[b]	0.44 ± 0.22[b]	0.22 ± 0.22[b]

Values are means followed by standard errors. Within each column, different letters indicate significant differences among treatments (ANOVA, Tukey's HSD, $P < 0.05$)

compounds currently proved effective against red poultry mites are highly volatile, and any acaricidal effects attributed to them might be temporary, lacking in long-term residual toxicity (George et al. 2008, 2009). From an environmental friendly viewpoint, such a lack of residual toxicity could be considered as beneficial. On the other hand, the effective toxicity and repellent action over time are a key feature for a botanical pesticide (Pavela and Benelli 2016b). In this framework, our results are promising, since the *A. sieberi* oil at 2% maintained its toxicity for at least 7 days after the treatment, at variance with a number of evidences reported by the earlier literature. For example, lavender essential oil did not showed prolonged toxicity against *D. gallinae*. When lavender-treated filter papers were used in toxicity assay immediately after impregnation, the mortality rates of *D. gallinae* were 66–90% but, if used 24 h after impregnation, the mortality rates of *D. gallinae* fell to 11% or less (George et al. 2008). Similar findings have been also reported for thyme essential oil (George et al. 2010).

Conclusions

Overall, this study firstly sheds light on the promising toxicity and repellent activity of α-thujone-rich *A. sieberi* essential oil on adults of the poultry red mite, *D. gallinae*. The residual toxicity tests also highlighted the prolonged toxicity of this essential oil, until 7 days from a single treatment. Further field assays with selected molecules (i.e., α-thujone, β-thujone, and camphor) from the *A. sieberi* essential oil are ongoing, testing them alone and in synergistic blends (see also Benelli et al. 2017b, c). Lastly, future research will focus on microencapsulation of the α-thujone-rich *A. sieberi* essential oil (Pavela 2016) as a suitable technique to maintain the oil toxicity and repellent effect in field conditions.

Compliance with ethical standards

Conflict of interest   The authors declare that they have no competing interests.

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