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# Essential oils: effects of application rate and modality on potential for combating northern fowl mite infestations

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**Abstract.** The northern fowl mite (NFM), *Ornithonyssus sylviarum* (Mesostigmata: Macronyssidae), is the primary blood-feeding ectoparasite found on poultry in the U.S.A. Three experiments were conducted *in vitro* to test the acaricidal properties of cade, garlic, lavender, lemongrass, pine and thyme essential oils against NFM, and to evaluate whether these effects are altered by adjusting oil application rates and application modality (direct vs. vapour contact). Applied at the rate of 0.21 mg/cm$^2$, the essential oils of cade, thyme, lemongrass and garlic resulted in higher NFM mortality at 24 h post-application than lavender and pine oils, and the untreated and ethanol-treated controls. Cade and thyme were the most consistent and fast-acting of the essential oils in terms of toxicity to NFM. Cade applied at 0.21 mg/cm$^2$ and 0.11 mg/cm$^2$ and thyme applied at 0.21 mg/cm$^2$ were effective in eliminating NFM within 2 h through direct contact. The modality of application did not affect the efficacy of cade and thyme essential oils. The results suggest that essential oils may be utilized as alternatives to chemical pesticides and could be used as fumigants for the control of NFM.

**Key words.** xxxx.

## Introduction

The northern fowl mite (NFM), *Ornithonyssus sylviarum*, is found on over 70 species of North American birds (Knee & Proctor, 2007). It is the primary ectoparasite affecting chickens raised on commercial farms in the U.S.A. (Axtell & Arends, 1990) and is also common in backyard flocks (Murillo & Mullens, 2016). Northern fowl mites live permanently on the host's feathers and travel to the surface of the skin to blood feed, which can result in dermatitis (Martin & Mullens, 2012). In laying hens, NFM infestations have been associated with economic losses because of decreases in egg production and individual egg weights (Mullens *et al.*, 2009). The mites are also a nuisance to farm workers who handle the birds, in whom they can cause skin irritation (Riley & Johannsen, 1938).

Spray application of synthetic pesticides is the most common approach to controlling NFM infestations (Mullens *et al.*, 2009). However, the continued use of these products may be constrained by issues of acquired resistance (Kim *et al.*, 2004; Fiddes *et al.*, 2005) and strict legislation (Chirico & Tauson, 2002). There is also increased consumer demand for natural products as a result, in part, of public perceptions and concerns about environmental impacts and the presence of chemical residues in eggs and meat products (Isman, 2000). Other proposed strategies for NFM control include the provision of dust baths with diatomaceous earth, kaolin clay and sulphur dioxide (Martin & Mullens, 2012), and biological control with the fungus *Beauveria bassiana* (Rassette *et al.*, 2011).

Plant-based essential oils show promise as alternatives to the traditional synthetic pesticides. Many essential oils have been

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categorized as 'generally recognized as safe' (GRAS) by the U.S. Food and Drug Administration (2014). They are environmentally non-persistent and degrade quickly (Isman, 2006). Importantly, these compounds and their chemical constituents are known to be toxic through direct contact or fumigation to several other species of mite (Isman, 2000; George *et al.*, 2009). Contact toxicity studies conducted in adult poultry red mites, *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae), reported that 50 of 56 oils tested at 0.35 mg/cm$^2$ (Kim *et al.*, 2004) and 10 of 50 oils tested at 0.21 mg/cm$^2$ (George *et al.*, 2010a) resulted in 100% mortality within 24 h. The essential oils of cade, garlic, lavender, lemongrass, Scots pine and thyme were shown to be toxic to poultry red mites when applied at 0.21 mg/cm$^2$. Essential oils of thyme and garlic have also been demonstrated as toxic to the poultry red mite (Ranjbar-Bahadori *et al.*, 2014). Similarly, spraying of 10% garlic juice directly on to birds resulted in a decrease in the number of NFM in laying hens (Birrenkott *et al.*, 2000).

The overall objective of this study was to identify the *in vitro* acaricidal effectiveness of cade, thyme, lemongrass, garlic, lavender and pine essential oils in achieving NFM mortality. Three experiments were conducted. Experiment 1 tested the toxic effects of the six essential oils at the application rate of 0.21 mg/cm$^2$. Experiment 2, conducted in a more controlled environment, confirmed the findings of Experiment 1 and evaluated how application rates (lower doses for the most potent oils and higher doses for the less potent oils) affect the acaricidal properties of cade, thyme, lemongrass and garlic, the essential oils deemed most promising when tested at 0.21 mg/cm$^2$. Finally, Experiment 3 assessed whether the effectiveness of the most potent oils identified in the previous two experiments would be altered by the application modality (direct contact vs. vapour contact).

## Materials and methods

### *Experiment 1. Relative efficacy of six essential oils against NFM*

The first experiment was conducted in a temperature-controlled room, at 27 ± 2 °C, 55 ± 10% relative humidity (RH) in the Department of Entomology, Purdue University (West Lafayette, IN, U.S.A.). To allow for comparison across studies, the methods approximated those employed in research on the use of essential oils against poultry red mites (Kim *et al.*, 2004; George *et al.*, 2010a, 2010b, 2010c). Petri dishes (4.5 cm in diameter, 1.5 cm in depth; Fisher Scientific Co. LLC, Pittsburgh, PA, U.S.A.) were fitted with filter paper (Whatman filter paper No. 2, 4.25 cm in diameter; Fisher Scientific Co. LLC). The treatments included an untreated paper (control), filter paper treated with 500 μL ethanol (ethanol control), and filter papers treated with a solution of one of six essential oils (cade, thyme, lemongrass, garlic, lavender, pine). All oils were sourced from New Directions Aromatics, Inc. (Missisauga, ON, Canada). Table 1 gives the nomenclature, country of origin and density (used to calculate the application rate) of each of the oils used in this study. The oils were dissolved in 500 μL ethanol to ensure their even dispersal over the filter paper and applied at 0.21 mg/cm$^2$. The dishes were then placed in a fume hood for 3 min to allow the ethanol to evaporate. The inner sides of the Petri dishes were covered with a thin layer of mineral oil (VWR International LLC, Radnor, PA, U.S.A.) to prevent the mites from escaping and 50 μL of distilled water was applied to each filter paper to maintain a humid environment within each dish. Groups of 10–15 adult NFM (using those that were darker in colour and avoiding smaller and paler individuals) were transferred to the centre of each dish using a 10/0 paint brush. The Petri dishes were then covered and sealed (Petri-Seal™; Carolina Biological Supply Co., Burlington, NC, U.S.A.), and placed in plastic boxes with moist paper towels to maintain humidity as higher humidity has been shown to increase mite survival (Chen & Mullens, 2008). Northern fowl mites were obtained from feathers plucked from around the vent areas of infested hens. The mites, along with the feathers, were placed in ziplock bags and transported to the Entomology Department at Purdue University. Mite mortality, calculated as the number of mites per dish that could not be stimulated to move divided by the total number of mites counted within the dish during that observation time, was assessed under a dissecting microscope at 2 h, 4 h, 8 h, 12 h and 24 h after initial exposure. Mites that walked into and became trapped in the mineral oil barrier were excluded from the calculation. Because of mite availability (NFM were obtained from a commercial laying hen facility within 24 h of each testing day) and mortality assessment time requirements, data collections took place on three separate days. Two replicates of each treatment were performed on each testing day, yielding a total of six replicates per treatment over the course of the experiment.

**Table 1.** Nomenclature, country of origin and density (used to calculate application rate) of the essential oils used in the study.

| Essential oil | Latin name | Country of origin | Density at 25 °C |
|---|---|---|---|
| Cade | *Juniperus oxycedrus* | Spain | 0.991 g/mL |
| Thyme | *Thymus vulgaris* | India | 0.916 g/mL |
| Lemongrass | *Cymbopogon flexuosus* | India | 0.896 g/mL |
| Garlic | *Allium sativum* | India | 1.083 g/mL |
| Lavender | *Lavandula angustifolia* | South Africa | 0.879 g/mL |
| Pine | *Pinus pinaster* | Hungary | 0.950 g/mL |

### *Experiment 2. Effects of essential oil application rate on NFM mortality*

The second experiment was conducted in a temperature- and humidity-controlled chamber in the Department of Animal Science, University of California, Davis. Mean ± standard deviation temperature and RH within the chamber were 27.13 ± 0.61 °C and 78.70 ± 3.31%, respectively. The mites used in Experiment 2 were obtained on the day of testing from feathers plucked from the vent areas of infested hens. Although the full history of infestation was unknown, the hens were reported to have had mites for at least 8 months. The last mite mitigation strategy (topical application of hand sanitizer with mineral oil) had been administered 4 months prior to this study.

Building on the results from Experiment 1, this subsequent experiment tested whether cade and thyme, the two most potent oils, would maintain their effectiveness at lower application rates, and whether increasing the application rate would improve the effectiveness of garlic and lemongrass essential oils, which were less toxic when applied at 0.21 mg/cm$^2$. Experiment 2 additionally replicated all treatments tested in Experiment 1, which enabled confirmation of the previous results while controlling for the methodological shortcomings of the initial experiment, including the variable humidity conditions and the potential for mites to become entrapped in the mineral oil. A total of 192 Petri dishes (16 treatments × 12 replicates) were prepared using methods similar to those in Experiment 1. In addition to the six treatments tested in Experiment 1, the essential oils of cade and thyme were tested at application rates of 0.11 mg/cm$^2$ and 0.05 mg/cm$^2$, and garlic and lemongrass were tested at application rates of 0.42 mg/cm$^2$ and 0.84 mg/cm$^2$. As previously described, each oil was diluted in 500 μL ethanol and applied to a filter paper. A group of 14–17 adult NFM was added to each Petri dish. Mineral oil was not applied to the inside of the dish. Instead, to prevent mites from escaping, the top edge of the bottom portion of each Petri dish was covered with a thin layer of Tangle-Trap® Sticky Coating (Tanglefoot®, Scotts Miracle-Gro Co., Marysville, OH, U.S.A.) before the dish was closed and sealed with Petri-Seal™. Mite mortality was determined under a dissecting microscope at 2 h, 4 h, 8 h, 12 h and 24 h. Two replicates of each of the 16 treatments were assessed on each testing day, yielding a total of 12 replicates per treatment over the course of the 3-week experiment.

*Experiment 3. Impact of exposure method (direct vs. vapour contact) on NFM mortality*

Experiment 3 was conducted concurrently with Experiment 2 and tested whether exposure to the vapours of cade and thyme essential oils, the two most toxic oils, would be as effective against NFM as exposure through direct contact. A cottonwool ball and an uncovered Petri dish fitted with filter paper were placed adjacent to one another in a plastic container (11.1 cm in diameter, 3.6 cm deep). Cade or thyme essential oil dissolved in ethanol (0.21 mg/cm$^2$) or ethanol alone (500 μL) was added to either the filter paper (direct contact assay) or the cottonwool ball (vapour contact assay). Neither the filter paper nor the cottonwool ball was manipulated in the untreated control assay. The containers were placed under the fume hood for 3 min to allow the ethanol to evaporate. As in Experiment 2, a thin layer of Tanglefoot® was applied to the top rim of the bottom portion of each Petri dish. The substance was additionally spread on the top rims of the large containers. A group of 14–17 adult NFM was placed in the centre of each Petri dish. The test containers were then covered and sealed. Mite mortality was assessed under a dissecting microscope at 2 h, 4 h, 8 h, 12 h and 24 h after the mites had been placed in the dishes. Two replicates of each treatment were evaluated on each testing day, yielding 12 replicates per treatment.

*Statistical analysis*

The mortality in treatment groups was corrected to take account of control mortality using Abbott's formula (Abbott, 1925). The following formula was used:

$$\frac{1-y}{x} \times 100 = percent\ \ corrected\ \ mortality$$

where $y$ = the percentage of living mites in the treated plate and $x$ = the percentage of living mites in the untreated control plate.

A repeated-measures block design was adopted for the three experiments of the study. Data were analysed using the PROC MIXED model in SAS Version 9.4 (SAS Institute, Inc., Cary, NC, U.S.A.), with the Petri dish as the experimental unit. The model considered treatments (essential oils), time intervals (2 h, 4 h, 8 h, 12 h, 24 h post-application) and their interactions as fixed effects and the replicate as a random effect. Before analysis, data were tested for normality using the UNIVARIATE procedure in SAS and a probability distribution plot. Data that were not normally distributed were transformed using log transformation before analysis to meet the assumptions of the analysis of variance (ANOVA). Reported differences are based on normalized data. Statistical differences are reported when $P$-values were ≤ 0.05. Data are presented as non-transformed least squares mean ± standard error of the mean.

## Results

*Experiment 1. Relative efficacy of six essential oils against NFM*

The results of Experiment 1 are presented in Table 2. There was an interaction between essential oil treatment and the time of mortality assessment ($P < 0.001$) (Table 2, Fig. 1A). At 2 h post-treatment, cade and thyme had resulted in significantly higher mortality (93.1 ± 3.40% and 95.6 ± 3.28%, respectively) compared with lemongrass (49.8 ± 13.18%), garlic (4.3 ± 2.72%), lavender (3.4 ± 2.40%), pine (3.3 ± 2.10%), the ethanol control (0.0 ± 0.00%) and the untreated control (0.0 ± 0.00%). At 4 h and 8 h post-treatment, cade, thyme and lemongrass caused greater NFM mortality than garlic, lavender, pine, and the ethanol-treated and untreated controls ($P < 0.001$) (Table 2). At 24 h post-treatment, the highest levels of mite mortality resulted from exposure to cade, thyme, lemongrass and garlic essential oils (100 ± 0.00%, 100 ± 0.00%, 97.2 ± 2.77% and 92.6 ± 3.33%, respectively). Mortality achieved by pine and lavender oils (51.9 ± 6.90% and 43.5 ± 9.35%, respectively) did not differ from that in the untreated and ethanol-treated dishes (38.7 ± 10.90% and 48.5 ± 13.41%, respectively) at 24 h post-exposure.

*Experiment 2. Effects of essential oil application rate on NFM mortality*

The results of Experiment 2, which included the replication of treatments used in Experiment 1, are presented in Table 3. The essential oils of cade, thyme, lemongrass and garlic resulted in high (> 94%) mite mortality at 24 h after exposure, regardless of application rate. The efficacy of the oils was affected by the application rate, oil type ($F_{15,854} = 286.34$, $P < 0.0001$) and exposure time ($F_{4,854} = 65.73$, $P < 0.0001$), and their interaction ($F_{60,854} = 2.76$, $P < 0.0001$). The essential oils of cade and thyme were confirmed as having the highest potency, each causing over 95% mortality within 2 h of exposure when applied at

**Table 2.** Results of Experiment 1. Effects of six essential oil treatments,∗ untreated filter paper control and ethanol-treated filter paper control on northern fowl mite (NFM) mortality.†

| | | Percentage mortality at time after application, LSM ± SEM‡ | | | | |
|---|---|---|---|---|---|---|
| Treatment | NFM adults, *n*§ | 2 h | 4 h | 8 h | 12 h | 24 h |
| Cade | 74 | $93.1 \pm 3.37^a$ | $98.8 \pm 1.11^a$ | $100.0 \pm 00.00^a$ | $100.0 \pm 00.00^a$ | $100.0 \pm 00.00^a$ |
| Thyme | 77 | $95.6 \pm 3.28^a$ | $100.0 \pm 00.00^a$ | $100.0 \pm 00.00^a$ | $100.0 \pm 00.00^a$ | $100.0 \pm 00.00^a$ |
| Lemongrass | 78 | $49.8 \pm 13.18^b$ | $78.5 \pm 13.17^b$ | $83.8 \pm 11.23^b$ | $83.7 \pm 8.33^b$ | $95.8 \pm 2.77^a$ |
| Garlic | 78 | $4.3 \pm 2.72^c$ | $8.7 \pm 4.41^c$ | $19.2 \pm 6.33^c$ | $36.6 \pm 11.50^b$ | $88.3 \pm 3.33^a$ |
| Lavender | 75 | $3.4 \pm 2.40^c$ | $5.1 \pm 2.50^c$ | $15.8 \pm 5.45^c$ | $7.4 \pm 5.14^c$ | $43.5 \pm 9.35^b$ |
| Pine | 85 | $3.3 \pm 2.10^c$ | $11.8 \pm 5.82^c$ | $14.2 \pm 5.32^c$ | $18.5 \pm 11.16^c$ | $51.9 \pm 6.90^b$ |
| Untreated control | 96 | $00.0 \pm 00.00^c$ | $00.0 \pm 00.00^c$ | $2.2 \pm 1.62^c$ | $9.7 \pm 6.11^c$ | $38.7 \pm 10.90^b$ |
| Ethanol-treated control | 88 | $00.0 \pm 00.00^c$ | $2.3 \pm 2.38^c$ | $7.2 \pm 5.47^c$ | $14.5 \pm 8.48^c$ | $48.5 \pm 13.41^b$ |

∗A mixture of ethanol (500 µL) and one of six essential oils applied at 0.21 mg/cm.
†Calculated by dividing the number of dead mites by the total number of mites in the Petri dish at the observation time.
‡Least squares means are expressed with ± standard errors obtained from original data; reported differences are based on normalized data after log transformation.
§Numbers of usable adult mites are summed across replicates (total: six replicates).
[a,b,c]Values followed by different letters within the same column (same observation time) are significantly different ($P > 0.05$).
LSM, least squares mean; SEM, standard error of the mean.

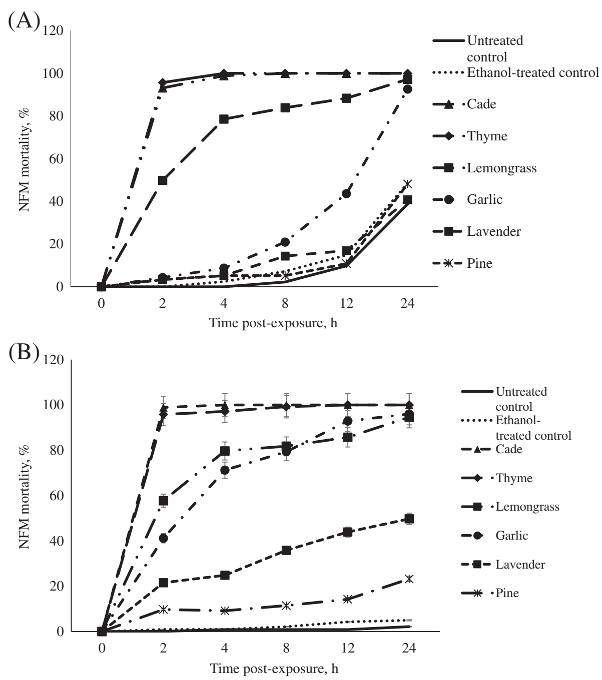


**Fig. 1.** Least squares mean ± standard error of the mean mortality in *Ornithonyssus sylviarum* subjected to six essential oil treatments applied at 0.21 mg/cm$^2$, and untreated control and ethanol control treatments at 0 h, 2 h, 4 h, 8 h, 12 h and 24 h post-exposure, showing an interaction between essential oil treatment and time of mortality assessment ($P < 0.001$). (A) Experiment 1. (B) Experiment 2.

0.21 mg/cm$^2$. After 4 h from initial exposure, lemongrass oil applied at higher doses (0.84 mg/cm$^2$ and 0.42 mg/cm$^2$) was more toxic to NFM than when applied at 0.21 mg/cm$^2$ and either of the controls ($P < 0.001$). From 4 h post-exposure, garlic oil tested at doses of 0.84 mg/cm$^2$ resulted in higher mortality than when applied at the lower rate of 0.21 mg/cm$^2$ and the control treatment ($P < 0.001$). The methodological changes that applied during the replication of Experiment 1 reduced the 24-h mortality in the untreated and ethanol-treated control groups from averages of 38.7% and 48.5% to 2.1% and 4.9%, respectively (Table 3, Fig. 1B).

*Experiment 3. Impact of exposure method (direct vs. vapour contact) on NFM mortality*

Table 4 summarizes the results of Experiment 3. Treatment ($F_{6,374} = 4162.93$, $P < 0.0001$) and time ($F_{4,374} = 8.77$, $P < 0.0001$), but not their interaction ($F_{24,374} = 0.72$, $P = 0.8346$), had an effect on NFM mortality. Application modality had no effect on the efficacy of the tested essential oils ($P > 0.05$) (Table 4). All essential oil treatments resulted in mortality of ≥ 91.6% within 2 h of application, which was significantly higher than that in the control groups ($P < 0.001$).

## Discussion

The results of this study identified cade, thyme, lemongrass and garlic essential oils as having the highest potential to serve as alternatives to synthetic pesticides in the control of NFM infestations. In contact toxicity tests (Experiments 1 and 2), all four oils resulted in high NFM mortality within 24 h of application, regardless of application rate. Oils from these plants were previously shown to be toxic to poultry red mites (*D. gallinae*) at 24 h after initial contact when applied at 0.21 mg/cm$^2$ (George *et al.*, 2010a) or 0.35 mg/cm$^2$ [Kim *et al.*, 2004 (garlic not tested)]. Contrary to the results of this study, pine (*Pinus sylvestris*) and some strains of lavender (*Lavandula officinalis*) were also reported as effective against poultry red mites (George *et al.*, 2010a). However, the pine oil used in the current study was of a different species and the lavender was sourced from a different geographic location. These differences

**Table 3.** Results of Experiment 2. Effects of different application rates on the efficacy of essential oils against the northern fowl mite (NFM).

| | | Percentage mortality at time after application, LSM ± SEM | | | | |
|---|---|---|---|---|---|---|
| Treatment* | NFM adults, *n*† | 2 h | 4 h | 8 h | 12 h | 24 h |
| Cade | | | | | | |
| 0.21 mg/cm$^2$ | 214 | 98.9 ± 4.75$^a$ | 100.0 ± 4.75$^a$ | 100.0 ± 4.75$^a$ | 100.0 ± 4.75$^a$ | 100.0 ± 4.75$^a$ |
| 0.11 mg/cm$^2$ | 204 | 89.4 ± 4.75$^a$ | 97.5 ± 4.75$^{ab}$ | 98.6 ± 4.75$^a$ | 99.0 ± 4.75$^{ab}$ | 100.0 ± 4.75$^a$ |
| 0.05 mg/cm$^2$ | 207 | 53.1 ± 4.75$^{bcd}$ | 68.8 ± 4.75$^{de}$ | 79.2 ± 4.75$^{cd}$ | 86.1 ± 4.75$^{bc}$ | 96.8 ± 4.75$^a$ |
| Thyme | | | | | | |
| 0.21 mg/cm$^2$ | 203 | 95.8 ± 4.75$^a$ | 97.2 ± 4.75$^{ab}$ | 99.24 ± 4.75$^a$ | 100.0 ± 4.75$^a$ | 100.0 ± 4.75$^a$ |
| 0.11 mg/cm$^2$ | 200 | 63.2 ± 4.75$^{bc}$ | 84.2 ± 4.75$^{abcd}$ | 91.8 ± 4.75$^{abc}$ | 95.4 ± 4.75$^{abc}$ | 99.6 ± 4.75$^a$ |
| 0.05 mg/cm$^2$ | 201 | 44.2 ± 4.75$^{cd}$ | 61.1 ± 4.75$^e$ | 75.4 ± 4.75$^d$ | 86.4 ± 4.75$^{abc}$ | 94.8 ± 4.75$^a$ |
| Lemongrass | | | | | | |
| 0.84 mg/cm$^2$ | 200 | 96.7 ± 4.75$^a$ | 100.0 ± 4.75$^a$ | 100.0 ± 4.75$^a$ | 100.0 ± 4.75$^a$ | 100.0 ± 4.75$^a$ |
| 0.42 mg/cm$^2$ | 200 | 68.8 ± 4.94$^b$ | 90.7 ± 4.94$^{abc}$ | 96.1 ± 4.94$^{ab}$ | 100.0 ± 4.94$^a$ | 100.1 ± 4.94$^a$ |
| 0.21 mg/cm$^2$ | 201 | 57.7 ± 4.75$^{bcd}$ | 79.6 ± 4.75$^{cd}$ | 81.79 ± 4.75$^{bcd}$ | 85.7 ± 4.75$^c$ | 94.6 ± 4.75$^a$ |
| Garlic | | | | | | |
| 0.84 mg/cm$^2$ | 208 | 69.1 ± 4.75$^b$ | 90.2 ± 4.75$^{abc}$ | 92.7 ± 4.75$^{abc}$ | 93.4 ± 4.75$^{abc}$ | 99.2 ± 4.75$^a$ |
| 0.42 mg/cm$^2$ | 216 | 58.3 ± 4.94$^{bcd}$ | 80.4 ± 4.75$^{bcd}$ | 87.9 ± 4.94$^{abcd}$ | 93.6 ± 4.94$^{abc}$ | 98.6 ± 4.94$^a$ |
| 0.21 mg/cm$^2$ | 200 | 41.4 ± 4.94$^d$ | 71.2 ± 4.75$^{de}$ | 79.6 ± 4.94$^{cd}$ | 93.1 ± 4.94$^{abc}$ | 96.0 ± 4.75$^a$ |
| Lavender 0.21 mg/cm$^2$ | 200 | 21.5 ± 4.91$^e$ | 24.8 ± 4.91$^f$ | 35.8 ± 4.91$^e$ | 43.9 ± 4.91$^d$ | 49.7 ± 4.91$^b$ |
| Pine 0.21 mg/cm$^2$ | 199 | 9.7 ± 4.90$^{ef}$ | 9.1 ± 4.90$^g$ | 11.4 ± 4.90$^f$ | 14.2 ± 4.90$^e$ | 23.2 ± 4.90$^c$ |
| Untreated control | 200 | 0.0 ± 4.94$^f$ | 0.8 ± 4.75$^g$ | 0.8 ± 4.75$^f$ | 0.8 ± 4.75$^f$ | 2.1 ± 4.75$^d$ |
| Ethanol-treated control | 201 | 0.9 ± 4.75$^f$ | 0.9 ± 4.75$^g$ | 2.1 ± 4.75$^f$ | 4.2 ± 4.75$^{ef}$ | 4.9 ± 4.75$^d$ |

*The density of each essential oil was used to calculate the amount of oil needed for each application (see Table 1).
†Numbers of usable adult mites are summed across replicates (total: 12 replicates).
[a,b,c]Values followed by different letters within the same column (same observation time) are significantly different ($P > 0.05$).
LSM, least squares mean; SEM, standard error of the mean.

**Table 4.** Effects of application method (contact vs. vapour toxicity) on northern fowl mite (NFM) mortality at an application rate of 0.21 mg/cm$^2$.

| | | Percentage mortality at time after application, LSM ± SEM* | | | | |
|---|---|---|---|---|---|---|
| Treatment* | NFM adults, *n*† | 2 h | 4 h | 8 h | 12 h | 24 h |
| Cade contact | 213 | 98.2 ± 1.79$^a$ | 99.3 ± 1.79$^a$ | 100.0 ± 1.79$^a$ | 100.0 ± 1.79$^a$ | 100.0 ± 1.79$^a$ |
| Cade vapour | 204 | 91.6 ± 1.79$^a$ | 97.6 ± 1.79$^a$ | 100.0 ± 1.79$^a$ | 100.0 ± 1.79$^a$ | 100.0 ± 1.79$^a$ |
| Thyme contact | 215 | 94.3 ± 1.79$^a$ | 96.5 ± 1.79$^a$ | 98.8 ± 1.79$^a$ | 100.0 ± 1.79$^a$ | 100.0 ± 1.79$^a$ |
| Thyme vapour | 205 | 91.7 ± 1.79$^a$ | 97.9 ± 1.79$^a$ | 100.0 ± 1.79$^a$ | 100.0 ± 1.79$^a$ | 100.0 ± 1.79$^a$ |
| Untreated control | 204 | 0.0 ± 1.79$^b$ | 0.8 ± 1.79$^b$ | 0.8 ± 1.79$^b$ | 0.8 ± 1.79$^b$ | 1.8 ± 1.79$^b$ |
| Ethanol-treated control (contact) | 206 | 1.0 ± 1.79$^b$ | 1.0 ± 1.79$^b$ | 4.0 ± 1.79$^b$ | 6.5 ± 1.79$^b$ | 6.5 ± 1.79$^b$ |
| Ethanol-treated control (vapour) | 204 | 0.7 ± 1.79$^b$ | 0.7 ± 1.79$^b$ | 2.3 ± 1.79$^b$ | 2.3 ± 1.79$^b$ | 2.3 ± 1.79$^b$ |

*Results are presented as the least squares means of untransformed data and reported differences are based on normalized responses after log transformation.
†Numbers of usable adult mites are summed across replicates (total: 12 replicates).
[a,b,c]Values followed by different letters within the same column (same observation time) are significantly different ($P > 0.05$).
LSM, least squares mean; SEM, standard error of the mean.

are likely to have contributed to the discrepancies between the studies. Differences in plant species or variety, the environment in which the plants were grown, and extraction and storage methods have been shown to affect the efficacy of some essential oils [reviewed by George *et al.* (2010a)]. Lavender oils, in particular, have yielded mixed results. The essential oil of lavender tested by Kim *et al.* (2004) resulted in high poultry red mite mortality (100% at 0.35 mg/cm$^2$ and 89% at 0.07 mg/cm$^2$) within 24 h of exposure. By contrast, George *et al.* (2010a) found that 24 h after initial exposure, poultry red mite mortality ranged from 75% to 100% across the six types of lavender oil tested. Another study showed no differences among the results produced by the same six lavender oils (George *et al.*, 2008). Based on the inconsistencies in results obtained in this and previous work, and the low mortality rates of NFM when pine and lavender were tested during Experiment 1, these two oils were deemed to be unreliably effective at best.

In view of the high mortality rates within the control groups observed in Experiment 1, all essential oils were re-tested at 0.21 mg/cm$^2$ during Experiment 2. Chen & Mullens (2008) demonstrated that the number of days that NFM survive off the host is impacted by temperature and humidity, and probably by the stage of infestation. George *et al.* (2010a) additionally noted that humidity and dust level have significant effects on the efficacy of some essential oils against red mites. The high control group mortality recorded in Experiment 1 may therefore reflect the fact that the NFM were collected on farms from hens of unknown, and potentially different, infestation stages and kept in a plastic ziplock bag for 24 h before the testing day, and the variation in RH within the test room over the course of the experiment. The use of mineral oils may also have resulted in a biased assessment of mite mortality as mites that became trapped in the oil were removed from the mortality count or were counted as dead. In order to control for these confounders, Experiment 2 was conducted in a temperature- and humidity-controlled chamber using mites obtained from a single flock on the day of testing. Mineral oil, which was applied to the inside of dishes in Experiment 1 to prevent mites from escaping as they were placed in the dishes, was replaced with Tangle-Trap® Sticky Coating applied to the top rim of the dish. Importantly, the main findings from Experiment 1 were unchanged. The essential oils of cade, thyme, lemongrass and garlic were confirmed as the most promising of the essential oils at 0.21 mg/cm$^2$, with cade and thyme having the fastest effects (Fig. 1B).

Overall, the present study identified cade and thyme essential oils as the most promising for use against NFM based on their fast activity against NFM and the consistency of results across all three experiments. Although both oils resulted in high NFM mortality within 24 h of application, their efficacy was affected by application rate. Cade essential oil achieved mortality of nearly 90% within 2 h when applied at 0.21 mg/cm$^2$ or 0.11 mg/cm$^2$, and within 12 h when applied at 0.05 mg/cm$^2$. Thyme essential oil was most rapidly effective at 0.21 mg/cm$^2$. Thyme and cade essential oils have previously been reported to maintain high potency against poultry red mites in contact toxicity tests across a number of temperature and humidity levels (George *et al.*, 2010a), and when applied at low doses of 0.07 mg/cm$^2$ and 0.04 mg/cm$^2$, respectively (Kim *et al.*, 2004; George *et al.*, 2008, 2010a). Thyme has also been shown to be effective in reducing poultry red mite numbers when sprayed directly on infested hens. A single treatment with thyme essential oil was reported to reduce mite infestation by 89% and 95% when evaluated at 1 day and 7 days after the initial spraying (Ranjbar-Bahadori *et al.*, 2014). It is worth noting that several plant species and sources of cade and thyme essential oils were used across the studies, which suggests an overall stability in the acaricidal properties of oils derived from these species of plant. A promising finding in the current study was that direct contact with the oils was shown to be unnecessary; this supports previous findings, which have suggested that essential oils are toxic to mites primarily through fumigant action (Kim *et al.*, 2004, 2007).

The essential oils of lemongrass (Kim *et al.*, 2004; George *et al.*, 2010a) and garlic (George *et al.*, 2010a) have also been shown to be toxic against poultry red mites. However, the application rate necessary to achieve mortality of > 90% has been shown to be greater than that for cade or thyme (George *et al.*, 2010a). Despite producing less stable results than cade and thyme, the essential oils of lemongrass and garlic do have the potential to serve as alternatives to traditional pesticides when used alone or in combination with other natural products. For example, Ranjbar-Bahadori *et al.* (2014) showed that spraying 0.07 mg/cm$^2$ garlic juice in a layer house infested with poultry red mites reduced the number of mites by 92% and 75% on days 1 and 7, respectively, after administration. Therefore, the acaricidal effects of lemongrass and garlic essential oils may be facilitated by combining these oils with other control strategies in an integrated pest management approach. Further research is needed to identify the effectiveness of the different chemical compounds in the essential oils that could be used as potential components of commercial acaricides.

Various studies, including this one, have demonstrated the potential of using essential oils to manage poultry red mite and NFM infestations. However, given the differences in the lifecycles of the two species, the optimal management strategies for combating the two mites are likely to differ. By contrast with poultry red mites, NFM live primarily on the host. Whereas management strategies aimed at controlling NFM should target the bird (via fumigation or topical treatment), those aimed at controlling poultry red mites should be applied to birds and their environments. When applied directly to a bird's skin, essential oils should be diluted with a carrier such as a vegetable or mineral oil to avoid skin irritations [e.g. sunflower oil has been used to dilute essential oils prior to application to cattle (Lachance & Grange, 2014)]. Additional studies are required to test for negative consequences to the bird and to determine the most effective mode of application (spray or fumigation).

The present findings lead to the conclusion that essential oils derived from cade, thyme, lemongrass and garlic are promising alternatives to synthetic pesticides for the control of NFM infestations. These results agree with those reported in studies of poultry red mites and NFM, which suggests that these oils may be successful in controlling many species of poultry mite and potentially other ectoparasites. Applications of lavender, lemongrass and pine essential oils to the skin of heifers have, for example, been reported as successful in repelling flies (Lachance & Grange, 2014).

The results highlight cade and thyme as the most potent of the tested oils. These oils were fast-acting and their acaricidal activity was stable across experiments. Their fumigant action against NFM was additionally confirmed. Using essential oils for the fumigation of poultry houses could provide a method of eradicating mites that are hiding in cracks and crevices (Mullens *et al.*, 2004). Further research is needed to evaluate the safety of these essential oils in terms of human and poultry health. Additional research is needed to investigate the effects of these essential oils on the different stages of NFM, although it is likely that their toxic effects will be similar in juvenile and adult mites, as has been shown in poultry red mites (George *et al.*, 2010b). The topical application of essential oils may be effective in controlling NFM infestations, especially for producers who market their eggs as organic or pesticide-free, and therefore warrants further research.

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