

## Control of the myiasis-producing fly, *Lucilia sericata*, with Egyptian essential oils

Hanem F. Khater<sup>1</sup>, PhD Abeer Hanafy<sup>2</sup>, PhD Abba D. Abdel-Mageed<sup>3</sup>, PhD Mohamed Y. Ramadan<sup>1</sup>, PhD, and Reham S. El-Madawy<sup>1</sup>, PhD

<sup>1</sup>Department of Parasitology, Faculty of Veterinary Medicine, Benha University, Benha, Egypt, <sup>2</sup>Department of Pharmacology, Faculty of Pharmacy, King Abdul Aziz University, Jeddah, Saudi Arabia, and <sup>3</sup>Department of Entomology, Faculty of Science, Benha University, Benha, Egypt

### Correspondence

Hanem F. Khater, PhD  
Department of Parasitology  
Faculty of Veterinary Medicine  
Benha University  
Moshtohor 13736,  
Egypt  
E-mail: hafkater@yahoo.com

Funding: None.

Conflicts of interest: None.

### Abstract

**Background** Myiasis caused by *Lucilia sericata* (Diptera: Calliphoridae) is widely distributed throughout the world and affects both humans and animals. In addition, *L. sericata* larvae and adults may play a role in spreading causal agents of mycobacterial infections. Therefore, it is important to establish new and safe alternative methods of controlling this blowfly.

**Methods** The insecticidal effectiveness of four commercially available essential oils [lettuce (*Lactuca sativa*), chamomile (*Matricaria chamomilla*), anise (*Pimpinella anisum*), rosemary (*Rosmarinus officinalis*)] against third larval instars of *L. sericata* was evaluated. The effects of sublethal concentrations of these oils on pupation rates, adult emergences, sex ratios, and morphological anomalies were also determined.

**Results** The oils were highly toxic to *L. sericata* larvae, with median lethal concentrations (LC<sub>50</sub>) of 0.57%, 0.85%, 2.74%, and 6.77% for lettuce, chamomile, anise, and rosemary oils, respectively. Pupation rates were markedly decreased after treatment with 8% lettuce oil, and adult emergence was suppressed by 2% lettuce and chamomile oils. Morphological abnormalities were recorded after treatment with all tested oils, and lettuce was the major cause of deformation. There was a predominance of males over females (4 : 1) after treatment with lower concentrations of chamomile and rosemary; such a skew toward males would lead to a population decline.

**Conclusions** The four tested oils are inexpensive and may represent new botanical insecticides for controlling blowflies.

### Introduction

Myiasis is a significant medical<sup>1–5</sup> and veterinary problem<sup>2,6,7</sup> that affects human welfare and national economies. Larvae of the green blowfly, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), are facultative ectoparasites that infest suppurative wounds. *Lucilia sericata* species are distributed throughout the world, infesting humans in America, Africa, and Asia.<sup>4</sup> Adult *L. sericata* can be found in unclean places, slaughterhouses, and butcher's shops.<sup>7</sup> Females lay eggs in the infected wounds of humans and animals, as well as in meat, fish, animal corpses, and excrement.<sup>1,7</sup> Nosocomial myiasis occurs in hospitalized patients; open wounds or lesions of bedridden patients may become infested if flies are in the environment.<sup>1,3</sup> Infestation and feeding activities of larvae at the skin surface can lead rapidly to the development of cutaneous lesions, further oviposition, debilitation, and death.<sup>6</sup> In addition, both larvae and adults of *L. sericata*

and *Calliphora vicina* (Diptera: Calliphoridae) can act as passive vectors of *Mycobacterium avium* sub *avium*, *Mycobacterium avium* paratuberculosis, and *Mycobacterium avium* hominissuis.<sup>8</sup>

*Lucilia sericata* is the species most used in maggot debridement therapy (MDT).<sup>9</sup> It has also been employed as an alternative to surgical intervention and long-term antiseptic therapy for the treatment of chronic wounds.<sup>10</sup> Sterile maggots should always be used because nonsterile maggots employed for MDT can cause deadly infections.<sup>11</sup> Maggots are capable of neutralizing a broad spectrum of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) in chronic wounds. However, under certain circumstances, maggots can serve as vectors for the transmission of pathogenic bacteria and thus should be promptly discarded after they have served their treatment role.<sup>10</sup> Debriding active pyoderma gangrenosum can result in wound enlargement (pathergy).<sup>12</sup> Maggot debridement therapy can be fatal if it is

performed on wounds that are likely to communicate with the central nervous system, a large blood vessel, body cavity, or vital organ.<sup>13</sup> Another potential complication of MDT is ammonia toxicity, which may lead to encephalopathy in patients with liver failure.<sup>9</sup>

Currently, the control of blowflies is accomplished mainly by organophosphate and synthetic pyrethroid dips and sprays,<sup>14–16</sup> as well as by insect growth regulators (IGRs).<sup>17,18</sup> However, these established methods are becoming ineffective because of growing resistance to insecticides.<sup>17,18</sup> To resolve the economic and environmental problems that result from the prolonged use of insecticides and IGRs, many have proposed the use of extracts from medicinal and herbaceous plants as safe alternatives for insect control.<sup>15,19–23</sup> Botanical insecticides are biodegradable and environmentally harmless.<sup>21</sup> Indeed, there is a long tradition of using essential plant oils as insecticides and repellents in homes and animal bedding. As natural and pleasant-smelling substances, they are highly acceptable to the public.<sup>24</sup> In addition, essential oils have antimicrobial effects on foods<sup>25</sup> and herpes simplex virus,<sup>26</sup> as well as wound-healing effects.<sup>27</sup>

Certain plant extracts have been effective in controlling *L. sericata*,<sup>28–31</sup> and applied oils such as anise,<sup>32–34</sup> chamomile,<sup>35</sup> and rosemary<sup>15,33,35</sup> are insecticidal against some species of medical and veterinary importance other than *L. sericata*.

*In vitro* assays are useful as screening processes before clinical and field applications are implemented.<sup>35,36</sup> The present study was designed to determine the insecticidal effects of oral treatment with four essential oils on third larval instars of *L. sericata*, using ingestion assays, as well as the effects of sublethal concentrations on pupation rates, adult emergences, sex ratios, and morphological anomalies.

## Materials and methods

Adult colonies of *L. sericata* were collected from Moshtohor, Qalyubia Governorate, Egypt, using fly netting. Flies were reared in the laboratory, according to El-Khateeb *et al.*<sup>28</sup> Four essential oils (obtained from El-Kaptain Co., Al-Obor, Cairo, Egypt) were tested, including: lettuce (*Lactuca sativa*); chamomile (*Matricaria chamomilla*); anise (*Pimpinella anisum*); and rosemary (*Rosmarinus officinalis*). The Egyptian Ministry of Health approved the oils for human use.

Procedures for the ingestion assays were performed according to Smith *et al.*<sup>37</sup> Early third larval instars of *L. sericata* were exposed to oils at five different concentrations: 0.05%, 0.5%, 2%, 4%, and 8% for lettuce; 0.1%, 0.3%, 1%, 2%, and 4% for chamomile; 1%, 2%, 6%, 12%, and 24% for anise; and 0.5%, 2%, 4%, 8%,

and 24% for rosemary. The oils were diluted in distilled water, and Tween-80 was added as an emulsifier. The procedures were replicated six times for each concentration of oil and an untreated control group. Ten larvae were used for each replicate (60 larvae were used for each concentration). Larvae were transferred to plastic cups, each of which contained 20 g of meat. One milliliter of the test material was then added. In the control groups, larvae were treated with 1 ml of water and Tween-80. Larvae were maintained under laboratory conditions at  $27 \pm 2^\circ\text{C}$ ,  $80 \pm 5\%$  relative humidity (RH), and a 16 : 8 h light : dark cycle. All assays were performed in triplicate. Larval mortality counts were determined daily until pupation. The developed pupae, at each concentration, were counted and placed in separate cages until adults emerged.

As a measure of the latent biological effects of the tested oils, pupation rates, adult emergences, and sex ratios were determined, as well as morphological deformities of larvae, pupae, and adults. Larval mortality counts were subjected to Probit transformation followed by regression analysis to determine lethal concentrations (LC<sub>50</sub>, LC<sub>90</sub>, and LC<sub>95</sub>) using the computer program POLO-PCO following Finney.<sup>38</sup> Statistical analysis was performed using SPSS Version 10 (SPSS, Inc., Chicago, IL, USA). Duncan's multiple range test was used for analysis of variance. Pearson's correlation coefficient and regression analysis were used to measure the degree of association between concentration and deformation of larvae, pupae, and adults.

## Results

The insecticidal efficacy of the tested oils increased as the concentration increased (Table 1). The sensitivity of *L. sericata* larvae to plant oils was demonstrated by LC<sub>50</sub> values of 0.57%, 0.85%, 2.74%, and 6.77% for lettuce, chamomile, anise, and rosemary oils, respectively. The slope functions were 1.174, 1.223, 1.342, and 1.219, respectively, illustrating homogeneous responses of *L. sericata* to the oils. Based on LC<sub>50</sub> values, the relative efficacy of the oils compared with that of rosemary (as a reference substance) indicated that lettuce, chamomile, and anise were 12, eight, and two times, respectively, more effective than rosemary.

Treatments of larvae conspicuously altered the biological parameters of pupation rate, adult emergence, and sex ratio (Tables 2 and 3). Pupation rates reached 6.67% and 10.00% after treatment with 8% lettuce and 24% anise oil, respectively. Adult emergence completely ceased following treatment of larvae with 2% lettuce and chamomile, 12% anise, and 24% rosemary oil. There was a predominance of males (ratio 4 : 1) after treatment with

**Table 1** The efficacy of essential oils on *Lucilia sericata* administered through ingestion assays

Applied oils	LC <sub>50</sub>	LC <sub>90</sub>	LC <sub>95</sub>	Relative efficacy	Slope
Lettuce	0.57	7.00	14.27	12	1.174 ± 0.222
Chamomile	0.85	9.47	18.76	8	1.223 ± 0.346
Anise	2.74	24.68	46.04	2	1.342 ± 0.405
Rosemary	6.77	76.31	151.60	1	1.219 ± 0.574

Data are given in concentration (%).

**Table 2** Effects of lettuce and chamomile essential oils on some biological factors of *Lucilia sericata*

Materials	Concentration, %	Pupation rate	Adult emergence	M : F	Larval deformity, %	Pupal deformity, %	Adult deformity, %
Lettuce	0.05	90.00 <sup>b</sup>	77.78 <sup>b</sup>	1.3 : 1	0.00 <sup>e</sup>	14.81 <sup>d</sup>	9.52 <sup>b</sup>
	0.5	50.00 <sup>c</sup>	26.67 <sup>c</sup>	1.0 : 1	13.33 <sup>d</sup>	26.67 <sup>c</sup>	50.00 <sup>a</sup>
	2	23.33 <sup>d</sup>	0.00 <sup>d</sup>	—	23.33 <sup>c</sup>	28.57 <sup>b</sup>	—
	4	13.33 <sup>e</sup>	0.00 <sup>d</sup>	—	40.00 <sup>b</sup>	50.00 <sup>a</sup>	—
	8	6.67 <sup>f</sup>	0.00 <sup>d</sup>	—	56.67 <sup>a</sup>	0.00 <sup>e</sup>	—
	Control	96.67 <sup>a</sup>	93.10 <sup>a</sup>	1.1 : 1	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>c</sup>
Chamomile	0.1	90.00 <sup>b</sup>	51.85 <sup>b</sup>	4.0 : 1	6.67 <sup>e</sup>	22.22 <sup>d</sup>	0.00
	0.3	70.00 <sup>c</sup>	42.86 <sup>c</sup>	3.5 : 1	16.67 <sup>d</sup>	28.57 <sup>c</sup>	0.00
	1	43.33 <sup>d</sup>	30.77 <sup>d</sup>	3.0 : 1	26.67 <sup>c</sup>	30.77 <sup>b</sup>	0.00
	2	30.00 <sup>e</sup>	0.00 <sup>e</sup>	—	30.00 <sup>b</sup>	33.33 <sup>a</sup>	—
	4	20.00 <sup>f</sup>	0.00 <sup>e</sup>	—	33.33 <sup>a</sup>	33.33 <sup>a</sup>	—
	Control	93.33 <sup>a</sup>	100.00 <sup>a</sup>	1.2 : 1	0.00 <sup>f</sup>	0.00 <sup>e</sup>	0.00

M, male; F, female.

Numbers within a column followed by the same superscript letter (a–f) are not significantly different ( $P > 0.05$ , Duncan's multiple range tests, SPSS software).

**Table 3** Effects of anise and rosemary essential oils on some biological factors of *Lucilia sericata*

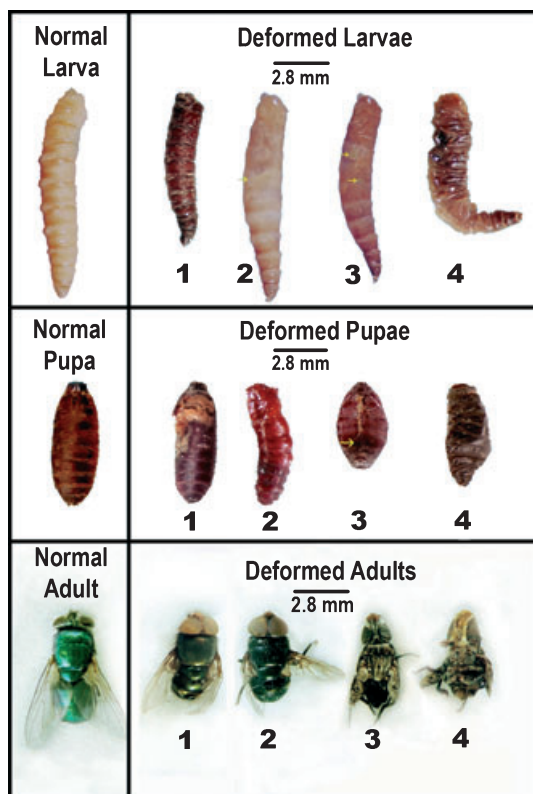
Materials	Concentration, %	Pupation rate	Adult emergence	M : F	Larval deformity, %	Pupal deformity, %	Adult deformity, %
Anise	1	66.67 <sup>b</sup>	90.00 <sup>b</sup>	1.3 : 1	6.67 <sup>d</sup>	5.00 <sup>d</sup>	5.56 <sup>c</sup>
	2	46.67 <sup>c</sup>	78.57 <sup>c</sup>	1.2 : 1	10.00 <sup>c</sup>	14.29 <sup>c</sup>	9.09 <sup>b</sup>
	6	30.00 <sup>d</sup>	77.78 <sup>c</sup>	1.3 : 1	13.33 <sup>b</sup>	22.22 <sup>b</sup>	14.29 <sup>a</sup>
	12	20.00 <sup>e</sup>	0.00 <sup>d</sup>	—	20.00 <sup>a</sup>	33.33 <sup>a</sup>	—
	24	10.00 <sup>f</sup>	0.00 <sup>d</sup>	—	0.00 <sup>e</sup>	33.33 <sup>a</sup>	—
	Control	93.53 <sup>a</sup>	93.33 <sup>a</sup>	1.0 : 0	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>
Rosemary	0.5	90.00 <sup>b</sup>	55.56 <sup>b</sup>	4.0 : 1	0.00 <sup>e</sup>	18.52 <sup>a</sup>	26.67 <sup>c</sup>
	2	73.33 <sup>c</sup>	22.73 <sup>c</sup>	4.0 : 1	13.33 <sup>d</sup>	13.64 <sup>b</sup>	40.00 <sup>a</sup>
	4	60.00 <sup>d</sup>	16.67 <sup>d</sup>	2.0 : 1	20.00 <sup>c</sup>	11.11 <sup>c</sup>	33.33 <sup>b</sup>
	8	43.33 <sup>e</sup>	15.38 <sup>e</sup>	1.0 : 1	26.67 <sup>b</sup>	7.69 <sup>d</sup>	0.00 <sup>d</sup>
	24	23.33 <sup>f</sup>	0.00 <sup>f</sup>	—	43.33 <sup>a</sup>	0.00 <sup>e</sup>	—
	Control	96.67 <sup>a</sup>	96.55 <sup>a</sup>	1.2 : 1	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>d</sup>

M, male; F, female.

Numbers within a column followed by the same superscript letter (a–f) are not significantly different ( $P > 0.05$ , Duncan's multiple range tests, SPSS software).

lower concentrations of chamomile and rosemary oils, but the ratios were not greatly changed after treatment with lettuce and anise oils.

Morphological abnormalities were pronounced after treatment with all four oils (Tables 2 and 3). Lettuce was the foremost cause of deformation (Fig. 1) in larvae



**Figure 1** Morphological abnormalities after treatment with essential plant oils. Larval abnormalities include: (1) a small, shrunk larva with diffuse brown pigment; (2) a weak cuticle with ulceration (arrow); (3) a weak cuticle with ulceration (arrows) and patches of brown pigment; and (4) a twisted larva with diffuse brown pigment. Pupal deformations include: (1) a puparium with an abnormal eclosion fissure; (2) a larviform puparium; (3) a small cracked puparium with a central groove (arrow); and (4) a small and distorted puparium. Adult anomalies include: (1) a deformed wing; (2) a deformed wing and legs; (3) a crumpled wing and deformed legs; and (4) a small, crumpled, poorly developed adult

(57%), pupae (50%), and adults (50%), resulting in small, shrunk, pigmented, and twisted larvae, as well as larvae with weak ulcerated cuticles. Pupal malformations included small, larviform, cracked, and distorted puparia. Adult deformities included small total size and deformities of the wings, abdomen, and legs.

In general, there was no significant relationship between the concentration of oil and deformation, except in four cases, for lettuce, chamomile, rosemary, and anise oils. A *P*-value of  $<0.05$  was considered statistically significant. The results showed a significant linear relationship between concentration and deformation of larvae treated with lettuce oil ( $r^2 = 0.973$ ; thus the concentration explains about 97.3% of the variation in deformation;

the simple linear equation is  $y = 5.218 + 7.012x$ ), chamomile ( $r^2 = 0.674$ ,  $y = 10.084 + 7.096x$ ), and rosemary ( $r^2 = 0.851$ ,  $y = 6.341 + 1.685x$ ). A significant relationship was also found for pupae exposed to anise oil ( $r^2 = 0.733$ ,  $y = 8.191 + 1.312x$ ).

## Discussion

Larvae of *L. sericata* induce myiasis in humans<sup>1-5</sup> and animals (especially domestic sheep)<sup>2,6,7</sup> and contribute to the spread of organisms that cause mycobacterial infections.<sup>8</sup> Our data demonstrate the insecticidal effects of four plant essential oils against *L. sericata*. Lethal effects increased as concentrations of oils increased, and inhibition of development occurred at sublethal concentrations. Notes for guidance published by the Working Party on the Efficacy of Veterinary Medicines (European Commission III/3682/92-EN)<sup>39</sup> indicate that the overall effectiveness of ectoparasiticides for treating infestation by diptera species should be between 80% and 100% and preferably greater than 90%. The efficacy of the applied oils meets these criteria.

Blowfly strike is currently controlled primarily by the prophylactic use of chemical insecticides (diazinon, high *cis*-cypermethrin, alpha-cypermethrin, deltamethrin), as well as IGRs (cyromazine, dicyclanil, diflubenzuron).<sup>14,16,17</sup> Despite the benefits of these agents, widespread use and the massive application of insecticides have resulted in pest resistance<sup>17,18</sup> and environmental pollution.<sup>40</sup> Myiasis<sup>41,42</sup> and other ectoparasites<sup>43,44</sup> may be treated successfully with ivermectin. However, ivermectin is associated with impaired male fertility in mice,<sup>45</sup> rats,<sup>46</sup> cattle,<sup>47</sup> and sheep,<sup>48</sup> and with neonatal toxicity in rats.<sup>49</sup> Healthcare providers now face a serious lack of new commercial insecticides.<sup>35</sup>

Plant extracts have been studied recently to determine their larvicidal, adulticidal, and repellent potential. Plant essential oils consist of numerous different, mostly volatile low molecular weight (LMW) terpenoids,<sup>24</sup> which influence the behavioral responses of pests, with the monoterpenoid components appearing most useful as insecticides or antifeedants.<sup>50</sup>

Low molecular weight terpenoids may be too lipophilic to be soluble in the hemolymph, a fluid in the body cavities and tissues of invertebrates, which, in arthropods, functions as blood and in some other invertebrates functions as lymph; after crossing the cuticle, the trachea is the proposed route of entry.<sup>51</sup> According to Priestley *et al.*,<sup>52</sup> most insecticides bind to receptor proteins in the insect and, in so doing, interrupt normal neurotransmission, which leads to paralysis and, subsequently, to death. Recent evidence<sup>52</sup> suggests that LMW terpenoids may also bind to target sites on receptors that modulate

nervous system activity. Ionotropic gamma aminobutyric acid (GABA) receptors, the targets of the organochlorine insecticides lindane and dieldrin, are modulated by LMW terpenoids with varied structures.<sup>52</sup>

The four applied oils tested in this study were highly effective against *L. sericata* larvae, with LC<sub>50</sub> values of 0.57%, 0.85%, 2.74%, and 6.77% for lettuce, chamomile, anise, and rosemary, respectively. These oils retarded larval growth at sublethal concentrations. Comparable results have been reported after treatment of *L. sericata* with fenugreek, celery, radish, and mustard oils (LC<sub>50</sub> 2.81%, 4.60%, 6.93%, and 7.92%, respectively)<sup>29</sup> and commercial neem extracts, Nivaar, Neem Azal T/S, and Bio Dux (LC<sub>50</sub> 0.4%, 1.3%, and 4%, respectively).<sup>28</sup> Lower concentrations of some extracts, such as American wormseed (*Chenopodium ambrosioides*) and thyme (*Thymus vulgaris*),<sup>31</sup> are effective against *L. sericata*, as are the volatile oils of dill (*Anethum graveolens*) and burnoof (*Conyza dioscoridis*).<sup>30</sup> Great retardation of *Parasarcophaga aegyptiaca* (Diptera: Sarcophagidae) larval development was caused by dill and burnoof oils (LC<sub>50</sub> were 70 ppm and 150 ppm, respectively).<sup>20</sup>

To understand why rosemary was the least toxic oil in our study, we employed ingestion assays and compared the lethal activities of oils using both a filter paper contact bioassay and a fumigation assay. We found that measurement of potency differs depending on test method. For example, rosemary, eucalyptus, and pennyroyal oils are more effective when tested in closed containers than in open ones, indicating that the effect of these oils is mainly related to the vapor phase, which has high fumigant toxicity.<sup>54</sup> Variation in assay results may reflect differences in the tested species, assay methods, and oil exposure times. In our study, treatment with sublethal concentrations adversely affected some biological aspects, including pupation rates and adult emergences. Such results have been observed in many myiasis-causing flies exposed to other oils, including fenugreek, celery, radish, and mustard oils,<sup>29</sup> as well as with neem extracts<sup>28</sup> against *L. sericata*, pomegranate (*Punica granatum*) against *Chrysomya albiceps* (Diptera: Calliphoridae)<sup>23</sup> and clove (*Eugenia aromatica*) and thevetia (*Thevetia peruviana*) oils against *P. aegyptiaca*.<sup>20</sup> In addition, sesame (*Sesamum indicum*), nigella (*Nigella sativa*), and onion (*Allium cepa*) oils adversely affect pupation and adult emergence rates of the housefly, *Musca domestica* (Diptera: Muscidae), and the house mosquito, *Culex pipiens* (Diptera: Culicidae).<sup>22</sup>

Our data show a preponderance of males over females (4 : 1) after treatment of larvae with sublethal oil concentrations, especially with chamomile and rosemary, which could lead to the demise of the *L. sericata* population. Analogous sex ratios have been recorded after treatment

of *L. sericata* with mustard oils (3 : 1), fenugreek and celery (2 : 1), and radish (1.5 : 1),<sup>29</sup> *P. aegyptiaca* with thevetia and clove oils (1.2 : 1),<sup>20</sup> *M. domestica* with not only plant oils of sesame (3.7 : 1), nigella (2.3 : 1), and onion (1.7 : 1) but also with IGRs diflubenzuron (5.0 : 1) and pyriproxifen (3.3 : 1),<sup>22</sup> and *Cx. pipiens* with onion (2.5 : 1) and sesame oils (2.3 : 1).<sup>22</sup> Morphological anomalies occurred after treatment with all tested oils and lettuce oil was the major cause of deformation. Similar malformations in myiasis-producing flies have been observed after treatment with several plant extracts, such as fenugreek, celery, radish, and mustard oils,<sup>29</sup> neem,<sup>28</sup> New Zealand gymnosperms,<sup>19</sup> pomegranate,<sup>23</sup> dill, burnoof, clove, and thevetia oils.<sup>20</sup> Comparable abnormalities have also been observed in *M. domestica* after treatment of third larval instars with sesame, nigella, and onion oils, and with IGRs diflubenzuron and pyriproxifen.<sup>22</sup> Abnormalities may be attributable to the metamorphosis-inhibiting effect of the plant oils, via the disturbance of hormonal control, which would suggest that plant oils have some influence on insect growth regulation.<sup>19,20,22,23,28,29</sup> The presence of larviform puparia may result from larvae that fail to contract to the pupal stage because of muscle paralysis, but the pupa cuticle melanizes because the enzymatic process of tanning continues.<sup>20</sup> Failure of adult emergences may be attributable to a combination of two or more of the following factors: unsaturated fatty acids accelerate the process of melanization and hardening of larvae (thus adults are unable to extricate from the pupal excuviae); there is insufficient pressure in the ptilinum, or hardening of the opercular suture occurs.<sup>20</sup>

Essential oils express insecticidal activities against several insects other than *L. sericata*. Extracts of rosemary have potential as natural larvicides and have shown adverse effects on pupae and adults developed from larvae of *Cx. pipiens* (LC<sub>50</sub> 6.58 ppm, 16.54 ppm, 19.15 ppm, and 43.75 ppm, respectively for acetone, hexane, ethanol, and diethyl ether extracts).<sup>15</sup> Rosemary oil reduces the hatchability of eggs (62.65%) and adversely affects some biological aspects of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae).<sup>55</sup>

Rosemary and chamomile oils had lethal effect against the buffalo louse, *Haematopinus tuberculatus* (Phthiraptera: Haematopinidae), for which LC<sub>50</sub> values of *in vitro* assays were 18.67% and 22.79%, respectively.<sup>35</sup>

Chamomile oil showed *in vitro* ovidical activity toward eggs of *H. tuberculatus*.<sup>35</sup> In addition, when used as a pour-on treatment, it significantly reduced the number of lice infesting buffaloes for four days post-treatment and expressed repellent effect for and flies infesting buffaloes, *M. domestica*, *Stomoxys calcitrans* (Diptera: Muscidae), *Haematobia irritans* (Diptera: Muscidae) and *Hippobosca equina* (Diptera: Hippoboscidae) for six days post-



treatment. No adverse effects were noted on either animals or pour-on operators after exposure to the applied oils.<sup>35</sup>

Anise oil is highly ovicidal and larvicidal, whereas rosemary oil is ovicidal and repellent against three Culicidae mosquito species, *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*.<sup>33</sup> Furthermore, anise oil demonstrated repellent effects against a wide range of insects, such as *Cx. pipiens*<sup>32</sup> and the human head louse, *Pediculus humanus capitis* (Anoplura: Pediculidae).<sup>34</sup>

Volatile oils reduce egg hatchability via the toxicity of oil vapors against eggs<sup>55</sup> or as a result of chemical ingredients which may diffuse into eggs, thus affecting vital processes associated with embryonic development.<sup>56</sup> The ovicidal and deterrent effects of anise,<sup>32,34</sup> rosemary,<sup>33</sup> and chamomile oils<sup>35</sup> could be utilized in prophylaxis against myiasis infestations. Studies have demonstrated the *in vitro* pediculicidal efficacy of some essential oils against female head lice that infest humans. Eucalyptus (*Eucalyptus globules*), rosemary, and pennyroyal (*Mentha Pulegium*, a member of the mint genus) oils were found to be at least as effective, if not more so, against *P. h. capitis* than *d*-phenothrin and pyrethrum, two commonly used pediculicides.<sup>53</sup> Essential oils, in particular, pennyroyal, tea tree and anise, have potent insecticidal activity against head lice and their eggs.<sup>24</sup> *Eucalyptus globulus* leaf oil-derived monoterpenoids are highly toxic to eggs and females of the human head louse.<sup>57</sup> Pennyroyal and its benzyl component are effective repellents against *P. h. capitis*.<sup>58</sup> Furthermore, essential oils contain monoterpenoids, which have lousicidal and ovicidal effects against *P. humanus*.<sup>52</sup> Peppermint and rosemary oils are reported to control such lice.<sup>51</sup>

Prevention of myiasis, especially hospital-acquired infestations, can be accomplished by maintaining clean dressings on wounds, placing screens over windows to prevent fly entry, and installing electrocutors on the walls of rooms and corridors to kill any flies that do enter.<sup>3</sup> For human safety, myiasis-producing flies should be controlled, especially around slaughterhouses and sheep farms.<sup>6,7</sup> Botanical control may be achieved by farm practices such as tail docking, shearing, appropriate control of gastrointestinal nematodes, and the removal of fecally soiled wool.<sup>14</sup> Larvae and adults of *L. sericata* can also spread causal agents of mycobacterial infections; this should be considered during sanitation processes in infected herds and in managing slaughterhouses in which the materials from animals affected by mycobacterial infections are processed.<sup>8</sup>

## Conclusions

The control of blowflies is important in terms of both human welfare and national economy but presents chal-

lenges concerning the identification of new, safe, and environmentally acceptable insecticides. The four essential oils tested in this study not only killed larvae when used at high concentrations but also induced morphological abnormalities that inhibited metamorphosis at sublethal concentrations. These oils can prevent adult emergence and protect against reinfestation. Further investigations into the efficacies of these oils may lead to the future development of effective natural blowfly control agents that could be integrated into other pest management programmes. Therefore, we recommend these essential plant oils for clinical and field evaluations.

## Acknowledgments

The authors are grateful to Dr Azza Moustafa, Research Institute of Medical Entomology, Cairo, Egypt, Dr Nagwa Ahmed, Parasitology Department, Faculty of Veterinary Medicine, Benha University, Benha, Egypt, and Dr Mohamed Hafez, Plant Pathology Department, Faculty of Agriculture, Benha University, Benha, Egypt, for their support and suggestions.

## References

- 1 Daniel M, Sramova H, Zalabska E. *Lucilia sericata* (Diptera: Calliphoridae) causing hospital-acquired myiasis of a traumatic wound. *J Hosp Infect* 1994; 28: 149–152.
- 2 Hall M, Wall R. Myiasis of humans and domestic animals. *Adv Parasitol* 1995; 35: 258–334.
- 3 Hira PR, Assad RM, Okasha G, et al. Myiasis in Kuwait: nosocomial infections caused by *Lucilia sericata* and *Megaselia scalaris*. *Am J Trop Med Hyg* 2004; 70: 386–389.
- 4 Service MW. *Medical Entomology for Students*. London; New York, NY: Chapman & Hall, 1996: 153–158.
- 5 Yaghoobi R, Tirgari S, Sina N. Human auricular myiasis caused by *Lucilia sericata*: clinical and parasitological. *Acta Med Iran* 2005; 43: 155–157.
- 6 Broughan JM, Wall R. Control of sheep blowfly strike using fly-traps. *Vet Parasitol* 2006; 135: 57–63.
- 7 Morsy TA, Fayad ME, Salama MM, et al. Some myiasis producers in Cairo and Giza abattoirs. *J Egypt Soc Parasitol* 1999; 21: 339–346.
- 8 Fischer OA, Matlova L, Dvorska L, et al. Blowflies, *Calliphora vicina* and *Lucilia sericata*, as passive vectors of *Mycobacterium avium* subsp. *avium*, *M. a. paratuberculosis*, and *M. a. hominissuis*. *Med Vet Entomol* 2004; 18: 116–122.
- 9 Sherman RA, Hall MJ, Thomas S. Medicinal maggots: an ancient remedy for some contemporary afflictions. *Annu Rev Entomol* 2000; 45: 55–81.

- 10 Daeschlein KY, Mumcuoglu O, Assadian B, *et al.* In vitro antibacterial activity of *Lucilia sericata* maggot secretions. *Skin Pharmacol Physiol* 2007; 20: 112–115.
- 11 Ruffi T, Steffen I, Nuesch R. Sepsis as a complication of maggot therapy. 5th International Conference on Biotherapy, 2004. Wurzburg.
- 12 Waterworth AS, Horgan K. Pyoderma gangrenosum – an unusual differential diagnosis for acute infection. *Breast* 2004; 13: 250–253.
- 13 Courtenay M, Church JC, Ryan TJ. Larva therapy in wound management. *J R Soc Med* 2000; 93: 72–74.
- 14 French NP, Wall R, Morgan KL. Ectoparasite control on sheep farms in England and Wales: the method, type and timing of insecticide application. *Vet Rec* 1994; 135: 35–38.
- 15 Shalaby AA, Khater HF. Toxicity of certain solvent extracts of *Rosmarinus officinalis* to *Culex pipiens* larvae (Diptera: Culicidae). *J Egypt German Soc Zool* 2005; 48: 69–80.
- 16 Tellam RL, Bowles VM. Control of blowfly strike in sheep: current strategies and future prospects. *Intern J Parasitol* 1997; 27: 261–273.
- 17 Levot G, Sales N. Insect growth regulator cross-resistance studies in field- and laboratory-selected strains of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). *Aust J Entomol* 2004; 43: 374–377.
- 18 Whyard S, Russell RJ, Walker VK. Insecticide resistance and malathion carboxylesterase in the sheep blowfly, *Lucilia cuprina*. *Biochem Genetics* 1994; 32: 9–24.
- 19 Gerard PJ, Ruf LD, Lorimer SD, Heath ACG. Activity of extracts and compounds from New Zealand gymnosperms against larvae of *Lucilia cuprina* (Diptera: Calliphoridae). *NZ J Agric Res* 1997; 40: 261–267.
- 20 Hussien KT. (1995) *Effect of some plant extracts in the control of a non-biting muscoid fly*. PhD thesis, Cairo University: Cairo.
- 21 Jacobson M. Insecticides from plants: a review of the literature. *Agricultural Handbook* 461. Washington DC: US Department of Agriculture 1975; 138: 1954–1971.
- 22 Khater HF. (2003) *Biocontrol of some insects*. PhD thesis, Zagazig University: Benha Branch, Benha.
- 23 Morsy TA, Mazyad SAM, El-Sharkawy IMA. The larvicidal activity of solvent extracts of three medicinal plants against third instar larvae of *Chrysomya albiceps*. *J Egypt Soc Parasitol* 1998a; 29: 91–100.
- 24 Williamson EM, Priestley CM, Burgess IF. An investigation and comparison of the bioactivity of selected essential oils on human lice and house dust mites. *Fitoterapia* 2007; 78: 521–525.
- 25 Gutierrez J, Barry-Ryan C, Bourke P. Antimicrobial activity of plant essential oils using food model media: efficacy, synergistic potential and interactions with food components. *Food Microbiol* 2009; 26: 142–150.
- 26 Koch C, Reichling J, Schnee J, *et al.* Inhibitory effect of essential oils against herpes simplex virus type 2. *Phytomed* 2008; 15: 71–78.
- 27 Jarrahi M, Vafaei AA, Taherian AA, *et al.* Evaluation of topical *Matricaria chamomilla* extract activity on linear incisional wound healing in albino rats. *Nat Prod Res* 2008; 22: 1197–1202.
- 28 El-Khateeb RM, Abdel-Shafy S, Zayed AA. Insecticidal effects of neem seed and vegetable oils on larval and pupal stages of sheep blowfly, *Lucilia sericata* (Diptera: Calliphoridae). *J Egypt Vet Med Assoc* 2003; 63: 255–268.
- 29 Khater HF, Khater DF. The insecticidal activity of four medicinal plants against the blowfly *Lucilia sericata* (Diptera: Calliphoridae). *Int J Dermatol* 2009; 48: 492–497.
- 30 Mazyad SA, El-Serougi AO, Morsy TA. The efficacy of the volatile oils of three plants for controlling *Lucilia sericata*. *J Egypt Soc Parasitol* 1999; 29: 91–100.
- 31 Morsy TA, Shoukry A, Mazyad SAM, Makled KA. The effect of volatile oils of *Chenopodium ambrosioides* and *Thymus vulgaris* against the larvae of *Lucilia sericata* (Meigen). *J Egypt Soc Parasitol* 1998b; 28: 503–510.
- 32 Erler F, Ulug I, Yalcinkaya B. Repellent activity of five essential oils against *Culex pipiens*. *Fitoterapia* 2006; 77: 491–494.
- 33 Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SPS. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. *Bioresource Technol* 2005; 96: 1749–1757.
- 34 Whitledge KL. Use of essential oils to repel and treat head lice. United States Patent 6342253; 2002. <http://www.freepatentsonline.com/6342253.html>. (Accessed 3 February 2010).
- 35 Khater HF, Ramadan MY, El-Madawy RS. The lousicidal, ovicidal, and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt. *Vet Parasitol* 2009; 164: 257–266.
- 36 Khater HF, Ramadan MY. The acaricidal effect of peracetic acid against *Boophilus annulatus* and *Argas persicus*. *Acta Sci Vet* 2007; 35: 29–40.
- 37 Smith KE, Wall R, Howard JJ, *et al.* In vitro insecticidal effect of fipronil and beta-cyfluthrin on larvae of the blowfly *Lucilia sericata*. *Vet Parasitol* 2000; 88: 261–268.
- 38 Finney DJ. *Probit Analysis: A Statistical Treatment of Sigmoid Response Curve*, 3rd edn. Cambridge University Press: Cambridge, 1971.
- 39 European Commission Working Party on the Efficacy of Veterinary Medicines. European Commission III/3682/92-EN. Commission of the European communities, Brussels.
- 40 Kumarasinghe SP, Karunaweera ND, Ihalamulla RL. A study of cutaneous myiasis in Sri Lanka. *Int J Dermatol* 2000; 39: 689–694.
- 41 Gassner B, Wüthrich A, Lis J, *et al.* Topical application of synthetic pyrethroids to cattle as a source of persistent environmental contamination. *J Environ Sci Health B* 1997; 32: 729–739.

- 42 Shinohara EH, Martini MZ, Oliveira Neto HG, *et al.* Oral myiasis treated with ivermectin: case report. *Braz Dent J* 2004; 15: 79–81.
- 43 Al-Eissa S, Gammaz HA, Hassan MF, *et al.* Evaluation of the therapeutic and protective effects of ivermectin and permethrin in controlling of wound myiasis infestation in sheep. *Parasitol Res* 2008; 103: 379–385.
- 44 Youssef MYM, Sadaka HAH, Eissa MM, *et al.* Topical application of ivermectin for human ectoparasites. *Am J Trop Med Hyg* 1995; 53: 652–653.
- 45 Conti-Díaz IA, Amaro J. Treatment of human scabies with oral ivermectin. *Rev Inst Med Trop Sao Paulo* 1999; 41: 259–261.
- 46 Otubanjo OA, Mosuro AA, Ladipo TF. An *in vivo* evaluation of induction of abnormal sperm morphology by ivermectin MSD (Mectizan®). *Pakistan J Biol Sci* 2007; 10: 90–95.
- 47 El-Nahas AF, El-Ashmawy IM. Effect of ivermectin on male fertility and its interaction with P-glycoprotein inhibitor (verapamil) in rats. *Environ Toxicol Pharmacol* 2008; 26: 206–211.
- 48 Avery B, Schmidt M. A dose–response study of ivermectin in a bovine *in vitro* production system. *Theriogenology* 1995; 43: 163–163.
- 49 Tanyildizi S, Bozkurt T. An investigation of the effects of ivermectin on blood serum, semen hyaluronidase activities and spermatological characteristics in sheep. *Turk J Vet Anim Sci* 2002; 26: 353–357.
- 50 Lankas GR, Minsker DH, Robertson RT. Effects of ivermectin on reproduction and neonatal toxicity in rats. *Food Chem Toxicol* 1989; 27: 523–529.
- 51 Palevitch D, Craker LE. Volatile oils as potential insecticides. *Herb Spice Med Plant Dig* 1994; 12: 1–5.
- 52 Veal L. The potential effectiveness of essential oils as a treatment for headlice. *Comp Ther Nurs Midwifery* 1996; 2: 97–101.
- 53 Priestley CM, Burgess IF, Williamson EM. Lethality of essential oils constituents towards the human louse *Pediculus humanus*, and its eggs. *Fitoterapia* 2006; 77: 303–309.
- 54 Yang YC, Lee HS, Clark JM, Ahn YJ. Insecticidal activity of plant essential oils against *Pediculus humanus capitis* (Anoplura: Pediculidae). *J Med Entomol* 2004a; 41: 699–704.
- 55 Moawad SS, Ebadah IMA. Impact of some natural plant oils on some biological aspects of the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). *Res J Agric Biol Sci* 2007; 3: 119–123.
- 56 Schmidt GH, Risha EM, Nahal AKM. Reduction of progeny of some stored product Coleoptera by vapors of *Acorus calamus* oil. *J Stored Prod Res* 1991; 27: 121–127.
- 57 Gurusubramanian G, Krishna SS. The effect of exposing eggs of four cotton insect pests to volatiles of *Allium sativum* (Liliaceae). *Bull Entomol Res* 1996; 86: 29–31.
- 58 Yang YC, Choi HY, Choi WS, *et al.* Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). *J Agric Food Chem* 2004b; 52: 2507–2511.
- 59 Toloza AC, Zygodlo J, Cueto GM, *et al.* Fumigant and repellent properties of essential oils and component compounds against permethrin-resistant *Pediculus humanus capitis* (Anoplura: Pediculidae) from Argentina. *J Med Entomol* 2006; 43: 889–895.