Bioactivities of some essential oils against the camel nasal botfly, Cephalopina titillator

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**Abstract** Nasopharyngeal myiasis of camels is caused by the larvae of *Cephalopina titillator*. We determined the efficacy of essential oils (EOs) of pumpkin, *Cucurbita maxima*; lupinus, *Lupinus luteus*; garlic oil, *Allium sativum*; and peppermint, *Mentha piperita*, against the third larval stage of *C. titillator* using larval immersion tests. The positive control group was treated with ivermectin and the negative control one was treated with distilled water and few drops of Tween 80. Larvae were reared until adult emergence. The data indicated that complete larval mortalities were reached 24 h post treatment (PT) with 2 % pumpkin, 7.5 % garlic and peppermint, 30 % lupinus, and 0.15 % ivermectin. The lethal values, LC50s, were 0.20, 0.44, 0.42, 0.47, and 0.03 %, respectively. Pumpkin and ivermectin were 2 and 17 times, respectively, more effective than the other EOs. Ivermectin was seven times more intoxicating than pumpkin oil. Formation of pupae had been stopped after treatment of larvae with 2 % pumpkin, 7.5 % garlic and peppermint, 30 % lupines, and 0.04 % ivermectin. Adult emergence had been completely ceased following treatment of larvae with 0.5 % EOs and 0.04 % ivermectin. Morphological abnormalities were pronounced after treatments, and peppermint oil was the foremost cause of deformation in larvae (44 % PT with 7.5 %) and pupae (40 % PT with 2 %). Pumpkin oil (6 %) was selected to be the drug of choice for controlling *C. titillator*. Besides their insecticidal effects, EOs are much safer than ivermectin regarding health and environmental issues. Consequently, EOs described herein merit further study as potential nasal drench for *C. titillator* control.

**Introduction**

Camels, *Camelus dromedarius*, are important species of livestock. In some areas of Egypt, people are used to consume camel's meat which has good quality and is economically fair in comparison to beef and mutton. Nasopharyngeal myiasis of camels is caused by the larvae of *Cephalopina titillator* (Clark 1797), an obligate parasite of the Oestridae family that attacks only camels (Hussein et al. 1982; Higgins 1985). During part of its life cycle, the female fly darts towards the nostrils and deposits its larvae directly into the nasal cavity. From there, the larvae crawl up to the nasopharynx and sometimes the paranasal sinuses and molt twice while attached to their mucous membranes. Maggots were also recorded in the frontal sinuses, nasal cavities, and tracheae of camel (Spratt 1984). They remain attached to the mucous membrane of these organs for up to 11 months causing extensive irritation and tissue damage. The mature third stage larvae (L3) grow up to 35 mm long. The mean larvae/camel was 28.45±6.48 (1–250) (Khater et al. 2013a). After maturation, L3 crawl back to the nasal passage and are expelled when the animal sneezes and then burrow into the soil for pupation.

The intensity of clinical signs depends on the number and damage caused by migrating larvae. Most infested camels developed clinical signs of nasal discharge, restlessness, loss of appetite, difficulty in breathing, frequent sneezing, and snoring (Zumpt 1965; Khater et al. 2013a). In sporadic cases, camels showed severe cough, ejection of soft palate and larvae, convulsions (Khater et al. 2013a) and abnormal behavior resembling cranial coenuriasis (Musa et al. 1989; Khater et al. 2013a), then camels may finally die from meningitis caused by secondary bacterial or viral infections (Musa et al. 1989). Infestations with the botfly impair animal welfare, reduce host physiological functions (El Bassiony et al. 2005a, b), destroy host tissues, and cause significant economic losses to livestock.
through reduction of milk production and weight gain (Hall and Wall 1995; Otranto 2001).

There are very few reports of the infections with the camel nasal botfly in the world. However, it is a common and widespread parasite where camels are found, for example, 41.67 % in Egypt (Khater et al. 2013a); 42.43 % in Iraq (Atiyah et al. 2011); 52 % in Saudi Arabia (Fatani and Hilali 1994); 74 % in Sudan (Steward 1950); 79 % in Libya (Abd El-Rahman 2010); and 80.72 % in Iran (Shakerian et al. 2011). Treatment of myiasis infestations has mainly based on systemic parasiticides as macrocyclic lactones (MCL), including ivermectin, doramectin, abamectin, and eprinomectin. Unfortunately, their use erased some safety and ecological crises (Lumaret and Errouissi 2002; El-Nahas and El-Ashmawy 2008; Seddiek et al. 2013). Consequently, a search for new alternatives for conventional insecticides is very crucial (Khater 2011, 2012, 2013; Khater et al. 2013a, b).


The ancient Egyptians may have been the first to discover the potential of essential oils (EOs) which developed also in the middle ages by Arabs. They are used in embalmment, in preservation of foods, and as antimicrobial, analgesic, sedative, antiinflammatory, spasmyloytic, and local anesthetic remedies. In recent times, EOs are produced commercially for pharmaceutical, sanitary, cosmetic, perfume, agricultural, and food industries, as food preservers and additives (Rajendran and Sriranjini 2008; Khater 2012, 2013).

Garlic possesses some biocidal effects as acaricidal (Aboelhadid et al. 2013); insecticidal (Kalu et al. 2010); anthelmintic (Mantawy et al. 2012); antiprotozoal (Ibrahim 2013); mouluscicidal (Mantawy and Mahmoud 2002); and antimicrobial and antifungal effects (Harris et al. 2001). Similarly, pumpkin has nematicidal (Klimpel et al. 2011); antiprotozoal (Grabensteiner et al. 2008; Elhadi et al. 2013); antidiabetic, antibacterial, hypocholesterolemic, antioxidant, anticancer, anthelmintic, antimutagenic, immunomodulatory, and antiinflammatory activities (Caili et al. 2006).

EOs are believed to act as allelopathic agents or as irritants that protect plants from predation by insects and infestation by parasites (Simpson 1995). EOs and their constituents have also been shown to be a potent source of botanical pesticides. They also adversely affect growth and development (Khater 2003; Shalaby and Khater 2005; Khater and Shalaby 2008; Khater et al. 2009, 2011; Khater 2003, 2012, 2013; Kumar et al. 2013) and alter feeding, mating, and oviposition behaviors (Khater 2011, 2012, 2013).

In addition, some of the applied oils, such as peppermint (Khani et al. 2012; Talbert and Wall 2012) and garlic (Yang et al. 2012; Mobki et al. 2013) show insecticidal effects. Because the applied oils are being tested against C. titillator for the first time, as far as we know, in vitro assays are useful to prescreen their efficacy. The aims of the present work were to determine the insecticidal effects of EOs of pumpkin, lupinus, garlic, and peppermint on L3 of C. titillator following dipping toxicity technique, and the effect of sublethal concentrations on certain biological parameters, such as pupation rates, adult emergence, and morphologic abnormalities.

Material and methods

Animals

The study was carried out on camels, 5–15 years old which brought originally for slaughtering from Sudan and Saini. Camels were slaughtered at Toukh’s slaughterhouse (35 km North Cairo), Qalyubia Governorate, Egypt, during the period from January to March, 2013. No information on prior parasitic treatment was available, but presence of parasitic fauna of infested animals and absence of dead larvae in inspected camel’s heads indicated that camels had not received any treatment for controlling C. titillator.

Collection of larvae

The camel’s heads were separated from the body after slaughtering. Each head was incised sagittally to expose the nasal and pharyngeal cavities and pharynx. The presence of L3 was checked, and then the recovered larvae were removed and identified in the laboratory according to Zumpt (1965).

Applied materials

- Ivermectin 1 % (Ivomec®, Merk Sharp and Dohme Agvet Inc.), applied for the positive control group.
- Four Egyptian oils, obtained from El-Captain Co., Al-Obor city, Cairo, Egypt, approved for human use from the Egyptian Ministry of Health. Such oils include the following: pumpkin, Cucurbita maxima; lupinus, commonly known as lupin or lupine, Lupinus luteus; garlic oil, Allium sativum; and peppermint, Mentha piperita.

Dipping technique

In vitro larval immersion (dipping) tests were carried out according to Khater et al. (2013a, b), to determine the efficacy of pumpkin, lupinus, garlic, and peppermint oils against L3 of C. titillator. Concentrations (60, 30, 7.5, 2, 0.5, 0.1, and 0.05 %) were freshly prepared in distilled water. Few drops of Tween 80 were added as an emulsifier to essential oils. The procedures...
were applied five times for each concentration and ten larvae of *C. titillator* were used per replicate in each test (i.e., 50 larvae were used for each concentration). Each group of larvae was placed in a mesh cloth piece and immersed for 60 s in a 100-ml solution of each material, and then the solution was continuously stirred during the process. The positive control group was treated with ivermectin (0.3, 0.15, 0.08, 0.04, 0.02, and 0.01 %) and the negative control one was treated with distilled water and few drops of Tween 80.

The immersed larvae were placed in Petri dishes having filter papers (Whatman No. 1) and then dishes were kept at 27±2 °C and 80±5 % relative humidity (RH). The mortality of larvae in all dishes was observed 6 and 12 h post treatment (PT).

Alive and dead larvae were counted. Larvae were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with wooden dowels; larvae which were incapable of movement, maintaining any signs of life, were considered moribund or dead (Khater and Ramadan 2007; Khater et al. 2013a, b).

Survived larvae were reared according to Hassanin et al. (1989). Each group of larvae were transferred to plastic cups (12.5 cm in diameter) containing sand (3–4 cm in depth) and then covered with a double layer of gauze. Cups were incubated at 26 °C until adult emergence. As a measure of the latent biological effects of the tested materials, pupation rates and adult emergences were determined, as well as morphological deformities of the developmental stages.

### Statistical analysis

Larval mortality counts were subjected to Probit transformation followed by regression analysis (Finney 1971) to determine lethal concentrations (LC values) using a computer program BioStat (version 2009 for windows, Build 5.8.4.3 © 2010 Analyst Soft Inc). The biological data were subjected to analysis of variance (ANOVA) with Duncan’s multiple range test (Duncan 1955) using a computer program (SPSS 16 for windows).

### Results

The insecticidal efficacy of the tested materials increased as the concentration increased. One hundred larval mortalities were reached 24 h PT with 2 % pumpkin, 7.5 % garlic and peppermint, 30 % lupinus, and 0.15 % ivermectin (Tables 1 and 2).

The sensitivity of *C. titillator* larvae to plant oils was demonstrated, 6 h PT, by LC50 values of 0.48, 1.2, 1.87, 2.18, and 0.05 % for pumpkin, lupinus, garlic, peppermint, and ivermectin, respectively. Moreover, the LC50 values, 24 h PT, were 0.20, 0.44, 0.42, 0.47, and 0.03 %, respectively (Table 3).

Based on LC50 values the relative efficacy of tested substances compared to that of peppermint (as a reference substance) indicated that pumpkin, lupinus, garlic, and ivermectin were 4, 2, 1, and 40 times, respectively, more effective than peppermint, 6 h PT. Pumpkin and ivermectin were 2 and 17 times, respectively, more effective than lupinus, garlic and peppermint, 24 h PT. Ivermectin was 9 and 7 times more intoxicating than pumpkin oil, 6 and 24 h PT, respectively (Table 3).

Treatments of larvae conspicuously altered some biological parameters such as pupation rate and adult emergence (Tables 4, 5, and 6). Formation of pupae had been stopped after treatment of larvae with 2 % pumpkin, 7.5 % garlic and peppermint, 30 % lupines, and 0.04 % ivermectin (Tables 4 and 6). Adult emergence had been completely ceased following treatment of larvae with 0.5 % EOs and 0.04 % ivermectin (Tables 5 and 6).

### Table 1 The efficacy of essential oils on *C. titillator* administered through larval immersion assays

<table>
<thead>
<tr>
<th>Conc. %</th>
<th>Concentration %; D. number of dead larvae; MO% mortality %</th>
<th>Time/h</th>
<th>60</th>
<th>30</th>
<th>7.5</th>
<th>2</th>
<th>0.5</th>
<th>0.1</th>
<th>0.05</th>
<th>--ve Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>D</td>
<td>MO%</td>
<td>D</td>
<td>MO%</td>
<td>D</td>
<td>MO%</td>
<td>D</td>
<td>MO%</td>
<td>D</td>
</tr>
<tr>
<td>Pumpkin</td>
<td></td>
<td>6</td>
<td>50</td>
<td>100.00a</td>
<td>50</td>
<td>100.00a</td>
<td>50</td>
<td>100.00a</td>
<td>38</td>
<td>76.00b</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>50</td>
<td>100.00a</td>
<td>50</td>
<td>100.00a</td>
</tr>
<tr>
<td>Lupines</td>
<td></td>
<td>6</td>
<td>50</td>
<td>100.00a</td>
<td>43</td>
<td>86.00b</td>
<td>39</td>
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<td>66.00c</td>
<td>25</td>
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</tr>
<tr>
<td>Peppermint</td>
<td></td>
<td>6</td>
<td>44</td>
<td>88.00a</td>
<td>41</td>
<td>82.00b</td>
<td>37</td>
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<td></td>
<td></td>
<td>24</td>
<td>50</td>
<td>100.00a</td>
<td>41</td>
<td>82.00b</td>
<td>37</td>
<td>74.00c</td>
<td>28</td>
<td>56.00d</td>
</tr>
<tr>
<td>Garlic</td>
<td></td>
<td>6</td>
<td>50</td>
<td>100.00a</td>
<td>41</td>
<td>82.00b</td>
<td>37</td>
<td>74.00c</td>
<td>28</td>
<td>56.00d</td>
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<tr>
<td></td>
<td></td>
<td>24</td>
<td>50</td>
<td>100.00a</td>
<td>50</td>
<td>100.00a</td>
<td>50</td>
<td>100.00a</td>
<td>39</td>
<td>78.00b</td>
</tr>
</tbody>
</table>

Values within a column followed by different lowercase letters were significantly different (P ≤ 0.05), while values within a column followed by the same lowercase letters were not significantly different (P ≤ 0.05)
Morphological deformities were pronounced after treatment with EOs as well as ivermectin (Tables 6 and 7), but peppermint oil was the foremost cause of deformation in larvae (44 % PT with 7.5 %) and pupae (40 % PT with 2 %). The aberrations include small, curved, pigmented, inflated, and distorted larvae as well as small, curved, distorted, and larviform (larval–pupal intermediate) pupae (Figs. 1 and 2).

**Discussion**

Our data designated that ivermectin was toxic to *C. titillator*. Complete larval mortalities were reached 24 h PT with 0.15 % ivermectin and the LC50, LC90, and LC99 were 0.05, 0.23, and 0.73 %, respectively, 6 h PT. To our knowledge, there are two studies that controlled *C. titillator* using MCL. Similar to our in vitro study, the mortality percentages of second stage larvae (L2) of *C. titillator* was 100 %, whereas those of L3 was 80 %, post dipping in 0.003 % doramectin. The lethal time, LT50, LT90, and LT99 values of doramectin, after treatment of L2 were 3.40, 13.13, and 39.51 h, respectively, and the corresponding values after treatment of L3 were 4.99, 22.95, and 79.66 h, respectively (Khater et al. 2013a). In addition, in vivo treatment with ivermectin was effective, 87.1 % (Robin et al. 1989).

Regarding treatment of the other obligate-myciasis-producing parasites, ivermectin was efficacious in the treatment of the cattle warble fly larvae, *Hypoderma bovis* (Alvinerie et al. 1994) and *Hypoderma lineatum* (Clyti et al. 2000); the goat warble fly infestation, *Przhevalskiana silenus* (Giangaspero et al. 2003); the New World screwworm, *Cochliomyia hominivorax* (Victoria et al. 1999); and the furunculic myiasis-producing larvae, *Dermatobia hominis* (Jelinek et al. 1995).

Despite the previous efficacy, ivermectin induces neonatal toxicity in rats (Lanks et al. 1989), and deleterious effects on the male fertility of cattle (Avery and Schmidt 1995), goats (Tanyildizi and Bozkurt 2002), and rats (El-Nahas and El-Ashmawy 2008). The toxic effects of IVR on liver (Ali et al. 1988; Seddiek et al. 2013) and kidney (Seddiek et al. 2013) functions were transient, and the treated rabbits required not less than 3 months after injection with ivermectin to regain their normality (Eman and Abdella 2000). More importantly, residues of ivermectin were found in muscle, liver, and milk (Galarini et al. 2013). Unfortunately, conventional cooking cannot be considered a safeguard against ingestion of residues of anthelmintic veterinary drugs in beef (Cooper et al. 2011). Furthermore, residues of ivermectin were stable in milk after 1 year of freezing at −20 °C; and they had diminished by approximately one quarter after 2 years of freezing (Cerkvenik et al. 2001). Consequently, it is not permitted to use ivermectin on dairy animals during lactation (Imperiale et al. 2004).

Myiasis producing maggots had been acquired resistance against organophosphorus insecticide (Carvalho et al. 2009). The documentation of ivermectin resistance had been reported (Currie et al. 2004; Levot and Sales 2008; Castro-Janer et al. 2011). Caution should also be exercised over any use of broad-spectrum compounds, such as ivermectin for myiasis control; as such applications will inevitably hasten the selection for resistance in gastrointestinal nematodes (Wall 2012).

Ecologically, most MCL have been shown to be highly toxic for the dung beetles, *Onthophagus taurus*, as a non-target (beneficial) organism (Wardhaugh et al. 2001; Lumaret and Errouissi 2002). Consequently, healthcare providers now face a serious lack of new alternative insecticides, which are urgently needed.

Botanicals could be an environment-friendly solution of the earlier dilemma (Khater 2011, 2012, 2013; Khater et al. 2013a). The work in the present study shed the light on in vitro controlling of the camel nasal botfly using some EOs. Our in vitro study signposted that 2 % pumpkin and 7.5 % garlic and peppermint oils generated 100 % mortality of *C. titillator* larvae. The LC50 values 6 and 12 h PT, were 0.48 and 0.02 %; 1.87 and 0.42 %; and 2.18 and 0.47 % for pumpkin, lupinus, garlic, and peppermint oils, respectively. Notes for guidance published by the Working Party on the Efficacy of Veterinary Medicines (European Commission III/3682/92-EN) indicate that the overall effectiveness of insecticides 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.

Using EOs as larvicides against *C. titillator* has not been done before, except for the previous analogous work of Khater.
Table 3  Lethal values±standard error of the applied materials

<table>
<thead>
<tr>
<th>Time/h</th>
<th>Pumpkin</th>
<th>Lupines</th>
<th>Garlic</th>
<th>Peppermint</th>
<th>Ivermectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
<td>24</td>
<td>6</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>LC</td>
<td>0.48±0.07</td>
<td>0.2±0.03</td>
<td>1.2±0.44</td>
<td>0.44±0.21</td>
<td>1.87±0.34</td>
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<tr>
<td>50</td>
<td>0.71±0.11</td>
<td>0.28±0.04</td>
<td>2.15±0.8</td>
<td>0.79±0.37</td>
<td>3.2±0.6</td>
</tr>
<tr>
<td>60</td>
<td>1.08±0.18</td>
<td>0.41±0.07</td>
<td>4.01±1.66</td>
<td>1.48±0.74</td>
<td>5.7±1.17</td>
</tr>
<tr>
<td>70</td>
<td>1.74±0.34</td>
<td>0.63±0.12</td>
<td>8.35±4.13</td>
<td>3.08±1.86</td>
<td>11.19±2.68</td>
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<tr>
<td>80</td>
<td>3.41±0.83</td>
<td>1.14±0.27</td>
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<td>8.49±6.98</td>
<td>28.55±8.58</td>
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<tr>
<td>90</td>
<td>5.93±1.7</td>
<td>1.86±0.54</td>
<td>53.45±43.03</td>
<td>19.61±20.73</td>
<td>61.87±22.19</td>
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<td>95</td>
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<td>4.7±1.82</td>
<td>257.92±298.93</td>
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<tr>
<td>99</td>
<td>RF1 4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
<td>RF2 2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>RF3</td>
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<td></td>
<td>RF4</td>
<td></td>
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</tbody>
</table>

Concentrations are represented in %

LC lethal values; SE standard error; RF1 relative efficacy of LC50 values, 6 h PT, according peppermint oil; RF2 relative efficacy of LC50 values, 24 h PT, according to peppermint oil; RF3 relative efficacy of LC50 values, 6 h PT, according Pumpkin oil; RF4 relative efficacy of LC50 values, 24 h PT, according Pumpkin oil.
et al. (2013a), as far as we know. After treatment for 24 h with 50 % lavender (Lavandula angustifolia), camphor (Cinnamomum camphora), and onion (Allium cepa) oils, the mortality percentages of L2 were 100, 80, and 52 %, respectively, whereas those of L3 were 100, 68, and 52 %, respectively. The LT50, LT90, and LT99 values of the same oils after treatment of L2 were 3.60, 12.06, and 32.31 h; 6.58, 51.67, and 106.51 h; and 9.28, 88.76, and 559.34 h, respectively. The correspondind values after treatment of L3 were 5.53, 18.71, and 277.38 h; and 14.24, 120.73, and 689.66 h, respectively. The LT50, LT90, and LT99 values of the same oils after treatment of L3 were 100, 80, and 52 %, respectively, two and four times less effective than doramectin and ivermectin.

Our data indicated that the relative efficacy, 6 h PT, indicated that pumpkin and lupinus were four and two times, respectively, more effective than garlic and peppermint. In addition, the relative efficacy of tested substances, 24 h PT, indicated that pumpkin and ivermectin were 2 and 17 times, respectively, more potent than peppermint, lupinus, and garlic oils. Ivermectin was 9 and 7 times more effective than peppermint, lupinus, and garlic oils. Ivermectin was 9 and 7 times more effective than pumpkin oil, 6 and 24 h PT, respectively. Similarly, the relative speed of efficacy indicated that lavender oil had similar potency as doramectin but camphor and onion oils were, respectively, two and four times less effective than doramectin and lavender (Khatjer et al. 2013a). It is possible that the assay methods, applied materials, and exposure time and concentration to test substances may be responsible for the variations between the assay outcomes.

Since we faced a shortage of literature about the used materials against C. titillator, we discussed our results with those for other insects, especially myiasis-producing flies. Obligate-miysias-producing flies were effectively controlled in few studies by EOs, such as camphor, Eucalyptus globulus, against the sheep nasal bot Oestrus ovis (Mazyad and Soliman 2001), and betel leaf (Piper betle) against the Old World screwworm, Chrysomya bezziana (Wardhana et al. 2007). Moreover, bovine hypodermias had been treated with the extract from dried tangerine peel (Wenqi et al. 1991).

In contrast to the obligate-miysias-producing flies, more work had been done for controlling the facultative ones. Larvae of the green blowfly, Lucilia sericata, infesting suppurrative wounds had been controlled by a variety of EOs, such as, fenugreek (Trigonella foenum-graecum), celery (Apium graveolens), radish (Raphanus sativus), and mustard (Brassica campestris) (Khatjer and Khatjer 2009); lettuce (Lactuca sativa), chamomile (Matricaria chamomilla), anise (Pimpinella anisum), and rosemary (Rosmarinus officinalis) (Khatjer et al. 2011); the American wormseed (Chenopodium ambrosioides) and thyme (Thymus vulgaris) (Morsy et al. 1998); and dill (Anthem graveolens) and burnoof (Conyza dioscoridis) (Mazyad et al. 1999).

**Table 4** Effects of the applied materials on the pupation of C. titillator

<table>
<thead>
<tr>
<th>Conc. %</th>
<th>No. %</th>
<th>No. %</th>
<th>No. %</th>
<th>No. %</th>
<th>No. %</th>
<th>No. %</th>
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</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.00d</td>
<td>0.00d</td>
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<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00d</td>
<td>15</td>
<td>30.00c</td>
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<tr>
<td>30</td>
<td>0.00f</td>
<td>0.00f</td>
<td>4.00f</td>
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<td>7.5</td>
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<td>0.00f</td>
<td>9.00f</td>
<td>18</td>
<td>18.00d</td>
<td>20</td>
<td>40.00d</td>
<td>29</td>
<td>58.00c</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>0.00f</td>
<td>0.00f</td>
<td>9.00f</td>
<td>18</td>
<td>36.00d</td>
<td>28</td>
<td>56.00d</td>
<td>35</td>
<td>70.00c</td>
<td>36</td>
</tr>
<tr>
<td>0.5</td>
<td>0.00f</td>
<td>0.00f</td>
<td>9.00f</td>
<td>18</td>
<td>36.00d</td>
<td>28</td>
<td>56.00d</td>
<td>35</td>
<td>70.00c</td>
<td>36</td>
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<tr>
<td>0.05</td>
<td>0.00f</td>
<td>0.00f</td>
<td>9.00f</td>
<td>18</td>
<td>36.00d</td>
<td>28</td>
<td>56.00d</td>
<td>35</td>
<td>70.00c</td>
<td>36</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conc. % concentration; No. Number of formed pupae; % pupation rate

Values within a column followed by different lowercase letters were significantly different (P ≤ 0.05), while values within a column followed by the same lowercase letters were not significantly different (P ≤ 0.05)

**Table 5** The effects of the essential oils on the adult emergence of C. titillator

<table>
<thead>
<tr>
<th>Adult emergence</th>
<th>Reduction %</th>
</tr>
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<tbody>
<tr>
<td>Conc. %</td>
<td>0.5</td>
</tr>
<tr>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>0.00c</td>
</tr>
<tr>
<td>Lupinus</td>
<td>0.00f</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.00f</td>
</tr>
<tr>
<td>Peppermint</td>
<td>0.00f</td>
</tr>
</tbody>
</table>

Conc. % concentration, No. Number of formed adult; % percentage of adult emergence

Values within a column followed by different lowercase letters were significantly different (P ≤ 0.05), while values within a column followed by the same lowercase letters were not significantly different (P ≤ 0.05)
In the same token, tea tree oil from *Melaleuca alternifolia* (terpinen-4-ol chemotype) induced insecticidal action against the Australian sheep blowfly, *Lucilia cuprina*, eggs and larvae, stimulating larvae to leave the wound (Callander and James 2012). New Zealand gymnosperms, such as *Podocarpus nivalis*, are effective against the first larval stage of *L. cuprina* (Gerard et al. 1997). Strong retardation of the larval development of *Parasarcophaga aegyptiaca* is caused by dill and barnoof extracts (Hussien 1995). Moreover, winged senna (*Cassia alata*) and betel leaf show larvicidal effects against *Chrysomya megacephala* (Kumarasinghe et al. 2002).

Our data revealed that the pupation rates and adult emergence were adversely affected after the treatment of larvae with sublethal concentrations of EOs. Comparable inhibitions were reported for pomegranate (*Punica granatum*) against *Chrysomya albiceps* (Morsy et al. 1998); and clove (*Eugenia aromatica*) and thevetia (*Thevetia peruviana*) oils against *P. aegyptiaca* (Hussien 1995); lettuce, chamomile, anise, and rosemary oils (Khater et al. 2011); fenugreek, celery, radish, and mustard oils (Khater and Khater 2009); and dill, barnoof, clove, and thevetia oils against *C. titillator*.

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### Table 6 The effect of ivermectin on some biological parameters of *C. titillator*

<table>
<thead>
<tr>
<th>Conc. %</th>
<th>0.04</th>
<th>0.02</th>
<th>0.01</th>
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</tr>
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<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Pupation</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Adult emergence</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>71.43</td>
</tr>
<tr>
<td>Adult reduction%</td>
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<td>75.00</td>
<td>47.50</td>
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</tr>
<tr>
<td>Malformations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae</td>
<td>16</td>
<td>32</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>Pupae</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Conc. % Concentration; No. Number of affected developmental stage**

Values within a column followed by different lowercase letters were significantly different (*P* ≤ 0.05), while values within a column followed by the same lowercase letters were not significantly different (*P* ≤ 0.05)

### Table 7 Malformations of *C. titillator* caused by essential oils

<table>
<thead>
<tr>
<th>Conc. %</th>
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<th>2</th>
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<th>0.1</th>
<th>0.05</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Larvae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pumpkin</td>
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<td>30</td>
<td>12</td>
<td>24</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Lupinus</td>
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<td>40</td>
<td>20</td>
<td>40</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
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<td>13</td>
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<td>11</td>
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<td>44</td>
<td>11</td>
<td>22</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Pupae</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pumpkin</td>
<td>0</td>
<td>-</td>
<td>0</td>
<td>0.00</td>
<td>6</td>
<td>23.08</td>
</tr>
<tr>
<td>Lupinus</td>
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<td>25</td>
<td>6</td>
<td>35.29</td>
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<td>26.92</td>
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<tr>
<td>Garlic</td>
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<td>-</td>
<td>5</td>
<td>29.41</td>
<td>6</td>
<td>31.58</td>
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<tr>
<td>Pepper</td>
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<td>-</td>
<td>8</td>
<td>40.00</td>
<td>9</td>
<td>29.03</td>
</tr>
</tbody>
</table>

**Conc. % Concentration; No. Number of malformed developmental stage**

Values within a column followed by different lowercase letters were significantly different (*P* ≤ 0.05), while values within a column followed by the same lowercase letters were not significantly different (*P* ≤ 0.05)
The presence of pupiform larvae and larviform pupae could be explained as larvae fail to contract to the pupal stage because of muscle paralysis, but the pupal cuticle melanizes because the enzymatic process of tanning continues (Hussien 1995). 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 (Hussien 1995).

Peppermint and some other EOs, such as camphor, onion, and chamomile pronounced ovicidal and pediculicidal activity against the buffalo louse, *Haematopinus tuberculatus* (Khater et al. 2009). EOs were also effective against the chewing louse, *Bovicola* (*Werneckiella*) *occellatus*, collected from donkeys. Such oils include peppermint, eucalyptus (*E. globulus* Labillardiere), clove bud (*Eugenia caryophyllata*), camphor (Talbert and Wall 2012), tea tree, and lavender (Talbert and Wall 2012; Ellse et al. 2013). Pennyroyal (*Mentha pulegium*, a species of mint) was proved to have potent acaricidal activity against the house dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Rim and Jee 2006). Exposure of freshly laid eggs of the spotted bollworm, *Earias vittella* (F.), to volatiles from the essential oils of peppermint and Japanese mint (*Mentha arvensis* L.) induce ovicidal and adversely affect pupal development (Marimuthu et al. 1997). EOs of peppermint, spearmint, pennyroyal oils were highly effective against the greenhouse whitefly, *Trialeurodes vaporariorum*, adults, nymphs, and eggs (Choi et al. 2003). Peppermint has been found to have fumigant toxicity against major stored product pests (Shaaya et al. 1991, 1997; Khani et al. 2012).

Garlic is an essential ingredient in the Arab cuisine. It is also considered to be a health food. However, garlic possesses various anti-insect properties. Garlic extracts were toxic to several species of mosquitoes (Denloye et al. 2003; Kimbaris et al. 2009; Kalu et al. 2010); ticks (Nchu et al. 2005; Costa-Júnior and Furlong 2011); stored product pests (Ho et al. 1996; Yang et al. 2012; Mobki et al. 2013); mushroom-infesting fly, *Lycoriella ingénue* (Park et al. 2006); and larvae of *Spodoptera littoralis* (Sadeghi et al. 2008), and the tobacco aphid, *Myzus nicotianae* (Sadeghi et al. 2007). Garlic also generates ovicidal effect against *Aedes aegypti* (Jarial 2001) and the stored product pest, *Tribolium castaneum* (Ho et al. 1996; Yang et al. 2010). The mode of actions of EOs has been reviewed in detail by Bakkali et al. (2008), Khater (2011, 2012, 2013), and Regnault-Roger et al. (2012). Plant extracts contain compounds that show ovicidal, repellent, antifeedant, sterilization, and toxic effects in insects (Isman 2006; Semmler et al. 2010; Al-Quraishy et al. 2012; Khater 2011, 2012, 2013; Khater et al. 2009, 2011).

![Fig. 1 Larval abnormalities (bar = 10mm). C Normal larva. 1 Small curved larva with dorsal inflation. 2 Small larva with distorted anterior end and enlarged tubercles. 3 Small larva with dark anterior projection. 4 Small larva with various malformed anterior end and enlarged tubercles. 5 Small larva with anterior projection, deformed tubercles, and inflation in the middle. 6 Larva with middle inflation at the ventral side. 7 Stumpy larva with middle inflation at the ventral side and dark pigmented and constricted area at the posterior third of the larva. 8 Pigmented pupiform larva with malformed anterior end and middle inflation and enlarged tubercles. 9 Small larva slightly pigmented with malformed tubercles and posterior end. 10 Small broad larva and its posterior end is broadly cut. 11 Small larva, stunted anteriorly and with enlarged abdominal segments posteriorly. 12 Curved pupiform larva. 13 Small pupiform larva, slightly pigmented, with inflation of the middle area, with malformed tubercles and with dorsiventrally flattened posterior end. 14 Slightly pigmented pupiform larva with pigmented anterior end and malformed tubercles](image-url)
complex mixture of components including minor constituents which act synergistically within the plant as a defense strategy. Hence, it is likely that they are more durable towards pests evolving resistance (Feng and Isman 1995).

The major constituents of the essential oil of garlic show insecticidal effect, such as thiosulphinates (Rajendran and Sriranjini 2008), methyl allyl disulfide, diallyl trisulfide (Huang et al. 2000), and diallyl disulfide (Park et al. 2006). Lectins that have been examined intensively for its toxic effect on insects is related to a lectin from the bulbs or leaves of garlic (A. sativum, ASA-II and ASAL) and the bulbs of onion (ACA) (Vandenborre et al. 2009). The lectin retarded the development of the larvae and their metamorphosis, and was also detrimental to the pupal stage resulting in weight reduction and lethal abnormalities. Plant lectins are carbohydrate-binding proteins that are incorporated in plant defense and found in many plant species (Peumans and Van Damme 1995). Lectins involved in the diet or cloned in transgenic plants have shown some insecticidal activity to various insect orders (Van Damme 2008), especially Lepidoptera, Coleoptera, Diptera, and Hemiptera. The main site of action for these compounds is the insect digestive system.

As repellents, extracts of garlic repel mosquitoes (Denloye et al. 2003; Campbell et al. 2011). Peppermint as well as camphor, onion, rosemary, and chamomile oils showed significant repellent activity against some nuisance flies (M. domestica, Stomoxys calcitrans, Haematobia irritans, and Hippobosca equina) infecting buffaloes in Egypt (Khater et al. 2009). Chopped garlic and garlic extracts repel beetles (Ho and Ma 1995). Moreover, some EOs are effective as alternative mosquito repellents, such as Zanthoxylum piperitum (Kamsuk et al. 2007) and Coriandrum sativum (Benelli et al. 2013).

Both diallyl disulfide and diallyl trisulfide, like garlic essential oil, acted as fumigants, produced behavioral deterrence and inhibited oviposition against angoumois grain moth, Sitotroga cerealella (Olivier) (Yang et al. 2012). Garlic is one of the potential plants that could be inserted in crops to decrease the pest occurrence in neighboring crop plots. Positively, both intercropping and application of volatile chemicals emitted by garlic could improve the population densities of natural enemies (Zhou et al. 2012). From the previously mentioned properties of EOs, it is expected that the applied oils, especially peppermint and garlic would kill eggs, repel adult flies, and stimulate attached larvae to leave their hosts. It is anticipated also that the antiinflammatory and antimicrobial properties of EOs would stimulate healing of wounds caused by the myiasis-producing maggots (Callander and James 2012).

In contrast to the safety and ecological issues associated to ivermectin, the use of EOs as low-risk insecticides has increased considerably owing to their popularity with organic growers and

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**Fig. 2** Pupal malformations (bar = 10mm). C Normal pupa. 1 Highly stunted pupa. 2 Small pupa with reddish tubercles. 3 Small pupa with failure of adult eclosion. 4 Larviform pupa. 5 Larviform pupa with broad and darkened anterior area and narrow and less pigmented posterior area. 6 Stunted larviform pupa. 7 Small pupa with narrow anterior end. 8 Narrow puparia with reddish brown exudates posteriorly. 9 Small curved puparia. 10 Small larva, body malformed anteriorly with some reddish brown tubercles posteriorly. 11 Slightly curve pupa with reddish brown tubercles anteriorly. 12 Pupa with reddish brown tubercles anteriorly and slight inflation posteriorly. 13 Broad pupa anteriorly with reddish brown tubercles anteriorly. 14 Small twisted puparia with reddish brown tubercles. 15 Short distorted and twisted pupa with diffuse reddish brown exudate anteriorly. 16 Small twisted pupa, compressed dorsoventrally. 17 Larviform pupa, dark anteriorly and unpigmented malformed posteriorly. 18 Larviform pupa with pigmented middle area and deformed posterior end.
environmentally conscious consumers. No adverse effects were noted on either animals or on operators prior to their exposure to the botanicals (Khater et al. 2009; Seddiek et al. 2013).

EOs and products based on them are safer to the users as they are mostly non-toxic to mammals, birds, and fish (Stroh et al. 1998; Isman 2000; Rajendran and Siriranjini 2008). Many of the commercial products that include EOs are on the generally recognized as safe (GRAS) list fully approved by the Food and Drug Administration and the Environmental Protection Agency in the USA for food and beverage consumption. Because they are also very close chemically to those plants from which they are derived, EOs are easily decomposed by microbes common in most soils and break down into harmless compounds within hours or days in the presence of sunlight (Rajendran and Siriranjini 2008; Khater 2013). In contrast to insecticides, non-target organisms such as predator, parasitoid, and pollinator insect populations will be less impacted on account of the minimal residual activity of EOs (Khater 2013).

Conclusions

As C. titillator adversely affected the health of infested camels, it should be safely controlled to reduce the use of chemical pesticides and also to avoid problems with insecticide resistance and residues. Pumpkin oil (6%) was selected to be the drugs of choice for controlling C. titillator. As it is not permitted to use MCL on dairy animals during lactation, ivermectin could be used to protect males, but EOs is much safer regarding health and environmental issues. Adult fly has two generations per year (Fatani and Hilali 1994; Alahme AM (2002) Seasonal prevalence of Cephalopina titillator larva in camels in Riyadh region, Saudi Arabia. Arab Gulf J Sci Res 20(3):161–164


Cephalopina titillator


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References


