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TOXICITY OF SOME ESSENTIAL OILS ON EGGS, LARVAE AND FEMALES OF _BOOPHILUS ANNULATUS_ (ACARI: IXODIDA: AMBLYOMMIDAE) INFESTING CATTLE IN EGYPT

BY SOBHY ABDEL-SHAFY\(^1\) and MAHMOUD M. M. SOLIMAN\(^2\)

( Accepted September 2003)

**SUMMARY:** Five essential oils: peppermint (_Mentha piperita_), spearmint (_Mentha viridis_), marjoram (_Marjorana hortensis_), lavender (_Lavandula officinalis_) and sweet basil (_Ocimum basilicum_) were tested against embryonated eggs, unfed larvae and fed females of the cattle tick _Boophilus annulatus_. _L. officinalis_ was more toxic on embryonated eggs (LC50: 0.36%) followed by _M. piperita_ (LC50: 0.39%), _O. basilicum_ (LC50: 0.52%), _M. hortensis_ (LC50: 1.06%) and _M. viridis_ (LC50: 1.20%). Toxicity of oils on unfed larvae decreased gradually from _O. basilicum_ (LC50: 0.05%) to _L. officinalis_ (LC50: 0.69%), _M. hortensis_ (LC50: 0.75%), _M. piperita_ (LC50: 0.77%) and _M. viridis_ (LC50: 0.90%). LC50 of _M. hortensis_ on fed females was 0.52% followed by _O. basilicum_, _M. piperita_, _L. officinalis_ and _M. viridis_ that were 1.01, 2.85, 3.25 and 10.57%, respectively. In conclusion these oils had toxic effects on all experimental stages of _B. annulatus_ except of _M. viridis_ was less toxic on fed females.

RÉSUMÉ : Cinq huiles essentielles (la menthe poivrée _Mentha piperita_, la menthe indigène, _Mentha viridis_, la marjolaine, _Marjorana hortensis_, la lavande _Lavandula officinalis_ et le basilic, _Ocimum basilicum_) ont été testées sur les œufs embryonnés, les larves à jeun et les femelles de _Boophilus annulatus_ en cours de gorgement sur le bétail. _L. officinalis_ s’est révélée la plus toxique pour les œufs embryonnés avec une concentration létale 50% (CL50) égale à 0,36%, inférieure à celles de _M. piperita_ (CL50 = 0.39%), _O. basilicum_ (CL50 = 0.52%), _M. hortensis_ (CL50 = 1.06%) et _M. viridis_ (CL50 = 1.20%). La toxicité des huiles pour les larves à jeun diminue progressivement de _O. basilicum_ (CL50 = 0.05%) à _L. officinalis_ (CL50 = 0.69%), _M. hortensis_ (CL50 = 0.75%), _M. piperita_ (CL50 = 0.77%) et _M. viridis_ (CL50 = 0.90%). La CL50 de _M. hortensis_ sur les femelles en cours de gorgement était de 0,52%, inférieure à celles de _O. basilicum_, _M. piperita_, _L. officinalis_ et _M. viridis_ qui étaient de 1,01; 2,85; 3,25 et 10,57%, respectivement. En conclusion, ces huiles ont des effets toxiques sur toutes les stases testées de _B. annulatus_. _M. viridis_ étant la moins toxique pour les femelles en cours gorgement.

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Ticks are important ectoparasites of domestic animals and affect about 800 million cattle and a similar number of sheep around the world (Kaya, 2000). Worldwide the economic losses caused by ticks and tick borne diseases in cattle are estimated at US $ 13.9 – 18.7 billion annually (De Castro, 1997). Using acaricides in control of ticks lead to many problems such as environmental pollution, development of resistant tick strains and escalating costs (Kaya, 2000). To save the high costs of importing acaricides and restrict other problems, its is important to search for the possibilities of using plants as sources of acaricides.

Extracts from various African plants have been shown to possess strong acaricidal and tick repel- lence properties. Some of the plant extracts are capable of reducing tick feeding, moulting, fecundity and viability of eggs (Kaya, 2000). Neem has been shown to possess anti-tick activities and to allow the control of the African ticks Rhipecephalus appendiculatus Neumann 1901, Amblyomma variega- tum (Fabricius, 1794) Boophilus decoloratus (Koch, 1844) (Kaya & Saxena, 1999) and of the Egyptian tick Hyalomma anatolicum excavatum Koch, 1844 sensu Pomerantsev, 1946 (Abdel-Shafy & Zayed, 2002).

Recently, it has been showed that essential oils derived from medicinal plants belonging to the family Lamiaceae have control effects on many pests and microorganisms. These oils have acaricidal (Per- rucci et al., 1996, Amer et al., 2001 & Momen et al., 2001); insecticidal (Keita et al., 2000 & 2001); bacte- ricidal (Koga et al., 1999 & Imai et al., 2001) and fungicidal effects (Montes Belmont & Carvaljal, 1998 and Rai et al., 1999).

In Egypt, the cattle tick B. annullatus (Say, 1821) was considered as the major tick species on cattle. It causes a reduction of cattle produc- tion and is vector of disease agents such as Babesia (Piroplasma) bigemina (Smith & Kilborne, 1893) and Babesia (Babesia) bovis (Babès, 1888) (Pro- tozoa, Apicomplexa, Sporozoaa, Piroplasmea = Piroplasmdia = Babesiiformes). Therefore our study aim at attempting to use five essential oils from plants belonging to the family Lamiaceae as an alternative method to chemical control of B. annullatus.

Material and Methods

1 – Ticks: The cattle tick, Boophilus annullatus was collected from Faculty of Agriculture farm, Cairo University, Giza. Ticks were collected from ground of cattle pens as fully fed females. Females were divided into two groups; one of them was treated with oils and another was incubated under 27° C, and 75% RH in the laboratory to obtain the eggs and larvae.

2 – Essential oils: Five essential oils from plants belonging to the family Labiatae were tested. These oils were peppermint (Mentha piperita); spearmint (Mentha viridis); marjoram (Marjorana hortensis); lavender (Lavandula officinalis) and sweet basil (Ocimum basilicum L.). Volatile oils were prepared from plants according to the method described by Momen et al. (2001). Plants were hydrodistilled for 3 hrs using a Clevenger-apparatus. The oils were separated and dried over anhydrous sodium sulphate. Emulsions of oils were prepared by mixing a few drops of Triton X on water. The concentrations applied on eggs and larvae were 0.5, 1, 2, 3, and 5% (M. piperita); 0.125, 0.25, 0.5, 1 & 2% (M. viridis, M. hortensis & L. officinalis) and 0.0625, 0.125, 0.25, 0.5 & 1% (O. basilicum). The concentrations used on females were 0.5, 1, 2, 3, and 5% (all oils).

3 – Treatments

3.1 – Embryonated eggs and unfed larvae: Five concentrations were prepared for each essential oil. Control was prepared with water and drops of Triton X. Each concentration and control included 5 replicates. Each replicate included 20 individuals in both eggs and larvae. Embryonated eggs and unfed larvae were dipped for 30 seconds in concentrations or control. Treated eggs and larvae were incubated under 27° C, and 75% RH. They were checked on the first and second day post application (J1, J2).

3.2 – Fed females: Five concentrations of each oil and control were prepared as in eggs and larval stages. Each concentration and control included 3 replicates. Each replicate included 10 fully fed females of B. annullatus. Females were treated, incubated and investigated as in egg and larval treatment.

4 – Statistical analysis and toxicity lines: Data were analyzed statistically by F and Tukey tests using SPSS computing program (1999). The percent of mortali-
ties were corrected for the natural mortality according to Abbott’s (1925) formula. Mortality curves were drawn on probit logarithmic graph paper according to the method developed by Finney (1971).

RESULTS

1 – Effects of essential oils on embryonated eggs. All oils had toxic effects on embryonated eggs of Boophilus annulatus. Mentha piperita oil caused mortality rates (68-100%) at concentrations varying from 0.5 to 5%. The concentrations from 0.125 to 2% of Mentha viridis, Marjorana hortensis and Lavandula officinalis led to mortality rates of 23 to 96%, 15 to 92% and 30 to 80%, respectively. The mortality rates of embryonated eggs ranged from 6 to 99% at concentrations of 0.0625 to 1% of Ocimum basilicum oil, Table (1).

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. piperita</td>
<td></td>
<td>M. viridis</td>
<td></td>
<td>M. hortensis</td>
<td>L. officinalis</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>2</td>
<td>96</td>
<td>92 b</td>
<td>80</td>
<td>1</td>
<td>99 b</td>
</tr>
<tr>
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<td>1</td>
<td>48</td>
<td>28 a</td>
<td>68</td>
<td>0.5</td>
<td>20 a</td>
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<td>99</td>
<td>0.5</td>
<td>47</td>
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<td>58</td>
<td>0.25</td>
<td>14 a</td>
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<tr>
<td>1</td>
<td>89</td>
<td>0.25</td>
<td>42</td>
<td>15 a</td>
<td>51</td>
<td>0.125</td>
<td>8 a</td>
</tr>
<tr>
<td>0.5</td>
<td>68</td>
<td>0.125</td>
<td>23</td>
<td>15 a</td>
<td>30</td>
<td>0.0625</td>
<td>6 a</td>
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<tr>
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<td>2 a</td>
<td>3</td>
<td>Control</td>
<td>5 a</td>
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<tr>
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<td>LC50</td>
<td>1.20</td>
<td>1.06</td>
<td>0.36</td>
<td>LC50</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Table 1: The effect of essential oils on embryonated eggs of Boophilus annulatus tick on the second day post application.

L. officinalis was more toxic (LC50: 0.36%) followed by M. piperita (LC50: 0.39%), O. basilicum (LC50: 0.52%), M. hortensis (LC50: 1.06%) and M. viridis (LC50: 1.20%). L. officinalis and M. piperita were more toxic for eggs than for larvae and females, Table (1) and Fig. (1). Dead eggs had a dark brown colour, were folded over, dried and no hatching occurred, Fig. (2).

2 – Effects of essential oils on unfed larvae: All essential oils had toxic effects on unfed larvae of B. annulatus. M. piperita oil (0.5-5%) led to mortality rates (30-97%). M. viridis, M. hortensis, and L. officinalis oils (0.125-2%) caused mortality rates of 15 to 74%, 6 to 84% and 26 to 83%, respectively. The mortality rates of unfed larvae ranged from 51 to 91% with O. basilicum oil (0.0625-1%) (Table 2).

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
<th>Concentration (%)</th>
<th>Mortality (%)</th>
</tr>
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<tbody>
<tr>
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<td>M. viridis</td>
<td></td>
<td>M. hortensis</td>
<td>L. officinalis</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>97</td>
<td>2</td>
<td>74 b</td>
<td>84 b</td>
<td>83 b</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>1</td>
<td>55 b</td>
<td>62 b</td>
<td>82 b</td>
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<td>27 a</td>
<td>42 a</td>
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<td>90</td>
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<td>0.25</td>
<td>16 a</td>
<td>19 a</td>
<td>26 a</td>
<td>0.125</td>
<td>79</td>
</tr>
<tr>
<td>0.5</td>
<td>30</td>
<td>0.125</td>
<td>15 a</td>
<td>6 a</td>
<td>26 a</td>
<td>0.0625</td>
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<td>0</td>
<td>0</td>
<td>Control</td>
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<td>42.7</td>
<td>53</td>
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<td>NS</td>
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<tr>
<td>LC50</td>
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<td>LC50</td>
<td>0.90</td>
<td>0.75</td>
<td>0.69</td>
<td>LC50</td>
<td>0.05</td>
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</tbody>
</table>

Table 2: The effect of essential oils on unfed larvae of Boophilus annulatus tick on 24 hrs post application.
<table>
<thead>
<tr>
<th>Conc. (%)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. piperita</td>
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<tr>
<td>5</td>
<td>98.3</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>1</td>
<td>13.3</td>
</tr>
<tr>
<td>0.5</td>
<td>6.7</td>
</tr>
<tr>
<td>Control</td>
<td>NS</td>
</tr>
<tr>
<td>LC50</td>
<td>2.85</td>
</tr>
</tbody>
</table>

Conc. = concentrations. NS = Non Significant. Tuk. = Tukey value

Table 3: The effects of essential oils on fed females of *B. annulatus* tick on the third day post application.

*O. basilicum* was more toxic on larvae (LC50: 0.05%) followed by *L. officinalis* (LC50: 0.69%), *M. hortensis* (LC50: 0.75%), *M. piperita* (LC50: 0.77%) and *M. viridis* (LC50: 0.90%). Generally, larvae were more susceptible than eggs and females specially in case of *O. basilicum*, *M. viridis* treatments (Table 2, Fig. 1).

![Graph showing LC50 values for different oils](image)

3. Effects of essential oils on fully fed females: All experiential oils had effects on fed females. Oil treatments (0.5-5%) caused mortality rates of 6.7 to 98.3%, 6.7 to 40%, 56.7 to 90%, 67 to 93.3% and 26.7 to 98% with *M. piperita*, *M. viridis*, *M. hortensis*, *L. officinalis* and *O. basilicum*, respectively, Table (3).

*M. hortensis* was more toxic (LC50: 0.52%) followed by *O. basilicum* (LC50: 1.01%), *M. piperita* (LC50: 2.85%), *L. officinalis* (LC50: 3.25%), and *M. viridis* (LC50: 10.57%), Table (3). Females were less susceptible to oil than eggs and larvae excepting of *M. hortensis* treatment, Fig. (1). Dead females were dark black-colored with high concentration (5%) and grey and yellowish-colored with low concentrations (0.5-3%) (Fig. 3).
Fig. 2. — The difference between eggs that responded to essential oils and untreated or insensitive (= normal) eggs of *Boophilus annulatus*: a) responded. b) normal.

Fig. 3. — The difference between females that responded to essential oils and untreated or insensitive (= normal) females of *Boophilus annulatus*: a) responded to 5% oils. b) responded to 0.5 to 3% oils. c) normal.
DISCUSSION

The tick Boophilus annulatus is a common tick and main vector of babesiosis on cattle in Egypt. It was controlled by chemical acaricides that are expensive and threatened by the widespread development of resistance. Furthermore, they caused environmental pollution and were toxic to mammals and humans. Therefore, it is necessary to search another alternative control method.

Recently, were utilized in tick-control plant derived materials such as: essential oils (Ndumu et al., 1999 and Lwande et al., 1999); Neem oil (Choudhury, 2001, Abdel-Shafy & Zayed, 2002 and Webb & David, 2002); crude plant extract (Kayaa et al., 1995 and Regassa, 2000) and plant-derived compounds (Bagherwal, 1999, Pand & Misra, 1997 and Kumer et al., 2000).

Our results indicated that all experiential oils had toxic effect on embryonated eggs, unfed larvae and fed females of B. annulatus tick. Larvae were more susceptible (LC50: 0.05-0.77%) followed by eggs (LC50: 0.35-1.68%) and females (LC50: 0.52-10.57%). Toxic effects of Lavandula officinalis and Mentha piperita were approximately equal on eggs and more potent than those of other oils. Toxicity of other oils decreased gradually from Mentha viridis and M. piperita that were approximately equal. In females M. hortensis was more toxic followed by O. basilicum, L. officinalis and M. piperita, however, the toxicities of the last two oils were approximately equal. M. viridis was less toxic on females, this result may attributed to the weak toxicity of the main compound of oil (carvone) or to the fact that it can not penetrate through the thick cuticle of females.

Toxicity of these oils is attributed to the presence of terpine compounds. These compounds differ from one oil to another. Amer et al. (2001) showed that M. hortensis oil had a toxic effect on two spider mites Tetranychus urticae Koch and Eutetranychus orientalis (Klein) (Acari: Actinedida: Tetranychidae). They also added that the main compounds of this oil were γ-terpinene and Sabinen + β-pinene. However, Refaat et al. (2002) revealed that O. basilicum and L. officinalis were effective on T. urticae and E. orientalis. They also referred to the main compounds of these oils: methyl chavicol and 1,8-cineol in O. basilicum and linalyl acetate and linalool in L. officinalis. Moreover, Momen et al. (2001) reported that M. viridis and M. piperita had toxicity on T. urticae. They also mentioned that carvone and D-limonene were the main compounds in M. viridis but M. piperita oil contained menthone and menthol as main compounds.

Channo et al. (2002) found that Eucalyptus camaldulensis oil had a toxic effect on the insects Sitophilus zeamais Motchulsky and Tribolium castaneum (Herbst) (Insecta: Coleoptera: Tenebrionidae). They also added that the toxic effect of this oil was attributed to the two major compounds, 1,8-cineol and terpinene. The compound 1,8-cineol was effective on larvae of T. castaneum (Tripathi et al., 2001). Menthol killed 100% of the mites Tyrophagus longior at the lowest dose (0.25 µl) by direct contact (Perrucci, 1995). About 0.2% menthone led to 37% mortality of larvae of the cutworm Peridroma saucia (Insecta: Lepidoptera: Noctuidae) (Harwood, et al., 1990).

In conclusion, these essential oils had toxic effect on eggs, larvae and females of B. annulatus except of that those M. viridis were less toxic on females. More studies are needed to confirm the acaricidal activity of these oils directly on animals.

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